IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Engelhardt et al.	)	
Serial No.:	08/479,997	)	Group Art Unit: 1634
Filed:	June 7, 1995	)	Examiner: Scott W. Houtteman
For:	OLIGO- OR POLYNUCLEOTIDES, AND OTHER COMPOSITIONS COMPRISING PHOSPHATE-MOIETY LABELED NUCLEOTIDES	)	

527 Madison Avenue (9th Floor)
New York, New York 10022
August 20, 2001

FILED BY EXPRESS MAIL

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Attention: Tech Center 1600

APPEAL BRIEF

I. REAL PARTY IN INTEREST

The real party in interest of the present application is Enzo Diagnostics, Inc., which is a subsidiary of Enzo Biochem, Inc. (hereinafter "Enzo"). Enzo is the owner of the present application by way of an assignment from the inventors, Engelhardt et al., of all rights, title, and interests.

II. RELATED APPEALS AND INTERFERENCES

There are no appeals or interferences related to the present application.

III. STATUS OF CLAIMS

All of the pending claims (claims 454-567) of the present application are rejected. A copy of the pending claims is attached hereto in the Appendix; specifically, Appendix

A contains the claims as amended by the Amendment submitted herewith and Appendix B contains the unamended claims.

IV. STATUS OF AMENDMENTS

After the Final Office Action dated July 18, 2000, the following were submitted by appellants: (1) an Amendment under 37 C.F.R. § 1.116 in Response to the July 18, 2000 Office Action dated January 18, 2001;[1] (2) an Information Disclosure Statement (IDS) dated March 15, 2001; (3) a Communication (To Provide Record of the Substance of the July 9, 2001, Interview) dated July 11, 2001; (4) a Supplemental After Final Amendment to Applicants' January 18, 2001 Amendment under 37 C.F.R. § 1.116 (And Following Their July 11, 2001 Communication) dated July 19, 2001;.

In an Advisory Action dated May 30, 2001, the January 18, 2001, Amendment was acted upon and the declarations and arguments submitted were considered. The amendments proposed in claims 454-567 were not entered.

The remaining submissions do not appear to have been acted upon by the Office. However, in the July 19, 2001, Supplemental Amendment, cancellation of claims 568-575 was proposed. Appellants anticipate that these amendments have been entered, as the cancellation of claims 568-575 places the application in better form for appeal by reducing and simplifying the issues for appeal. Furthermore, during a telephone conversation between the Office and Appellants' undersigned attorney Ronald C. Fedus, Examiner Scott W. Houtteman indicated that an after final

[1] Included with this amendment were the following: a Declaration of Dr. Cheryl H. Agris, Attorney at Law (In Support of the Written Description, Enablement & Non-Obviousness of the Invention Claimed in U.S. Patent Application Serial No. 08/479,997) and a Declaration of Dr. Ann Sodja (In Support of the Non-Obviousness of the Invention Claimed in U.S. Patent Application Serial No. 08/479,997).

amendment to cancel claims 568-575 would be entered. (*See* Supplemental Amendment – July 19, 2001, at 4.)

Appellants also conducted an Examiner Interview on July 9, 2001. In this interview, the Office withdrew the obviousness rejection under 35 U.S.C. § 103 over U.S. Patent No. 4,378,458 (Gohlke et al.) in view of Sodja et al., *Nucleic Acids Res.*, 5: 385-401 (1978) set forth in paragraph 5 of the final Office Action. (*See also*, Communication – July 11, 2001, at 9.)

For the convenience of the Board, attached hereto in the Appendix is a copy of the cited prosecution documents.

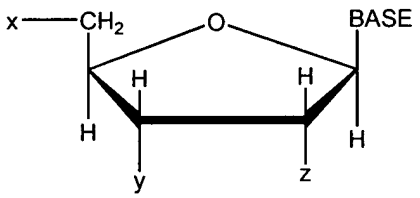
V. SUMMARY OF INVENTION

The present invention is directed to nucleic acid probes. Specifically, the claimed probes are oligo or polynucleotides (deoxyribonucleotides and ribonucleotides) that include at least one modified nucleotide in which a non-radioactively detectable label (Sig) is covalently attached to the phosphate moiety PM of at least one of the nucleotides of the oligo or polynucleotide either directly or via a chemical linkage. Further details of the claimed embodiments of the present invention are discussed below.

In one embodiment, the oligo or polynucleotide comprises at least one deoxyribonucleotide, or alternatively, at least one ribonucleotide, having the formula SIG—PM—SM—BASE. (Specification p. 93, ll. 1-11; Original Claims 1-8.) In this formula, PM is a phosphate moiety, SM is a sugar moiety, and BASE is a pyrimidine, a purine, or a deazapurine, or analogue thereof. (*Id.*) Sig is a label that is non-radioactively detectable when attached to PM or when the nucleotide is incorporated into the oligo or polynucleotide. (Specification p. 93, ll. 14-21; Original Claims 1-8.) PM

is attached to SM, BASE is attached to SM, and Sig is covalently attached to PM directly or via a chemical linkage. (Specification p. 95, ll. 2-10; Original Claims 1-8.) Provided that, when the formula encompasses a ribonucleotide and Sig is attached through a chemical linkage to a terminal PM at the 3' position of a terminal ribonucleotide, the chemical linkage is not a cleaved 3' terminal ribonucleotide previously attached to the oligo or polynucleotide.

In another embodiment, the oligo or polynucleotide comprises at least one deoxyribonucleotide, or alternatively, at least one ribonucleotide, having the structural formula:



(Specification p. 2, ll. 1-27.) In this formula, the BASE moiety is a pyrimidine, a purine, a deazapurine, or an analog thereof, wherein BASE is attached at the 1' position of the pentose ring from the N1 position when BASE is a pyrimidine or from the N9 position when BASE is a purine or a deazapurine. (Specification p. 3, ll. 1-5.) X is (i) H—, (ii) HO—, (iii) a mono-phosphate, (iv) a di-phosphate, (v) or a tri-phosphate. (Specification p. 19-21.) Y is (i) H—, (ii) HO—, (iii) a mono-phosphate, (iv) a di-phosphate, (v) or a tri-phosphate. (*Id.*) Z is H—. (*Id.*) Sig is non-radioactively detectable when attached to x, y, or z. (Specification p. 3, ll. 7-11.) Sig is covalently attached via a phosphate moiety to x, y, or z directly or via a chemical linkage. (Specification p. 3, ll. 13-18.) In another words, Sig can be attached to x, y, and/or z when x, y, and/or z are phosphates. Accordingly, x, y, and/or z can be H— or HO— when at least one other of the three groups is a phosphate to which the Sig label is attached. Provided that, when the

formula encompasses a ribonucleotide and Sig is attached through a chemical linkage to y, the chemical linkage is not a cleaved 3' terminal ribonucleotide previously attached to the oligo or polynucleotide.

Sig can be, or can render the nucleotide, self-signaling or self-indicating or self-detecting. (Specification p. 82, ll. 1-5; Original Claim 141, 143.) Sig can also comprises at least three carbon atoms. (Original Claim 13.) Further, Sig is covalently attached to the PM through the phosphate or phosphorus oxygen. (Specification p. 95, ll. 2-10.)

The PM of the present invention can be a mono-phosphate, a di-phosphate, or a tri-phosphate.

The chemical linkage attaching Sig to the oligo or polynucleotide does not interfere substantially with the characteristic ability of Sig to form a detectable signal. (Specification p. 96, ll. 22-28.) The chemical linkage can comprise an olefinic bond at the alpha-position relative to the point of attachment to the nucleotide, a —CH_2NH— moiety, or both. (Specification p. 3, ll. 24-29.) Further, the chemical linkage can comprise or include an olefinic bond at the delta-position relative to the point of attachment to the nucleotide, or any of the moieties (1) —$CH{=}CH_2$—NH—, (2) —CH=CH—CH_2—NH—, (3) —CH=CH—CH_2—O—CH_2—CHOH—NH—, (4) —S—, (5) —COO—, or (6) —O—. (*Id.*) The chemical linkage can also comprise an allylamine group. In another alternative, the chemical linkage can include a glycosidic linkage moiety. (Original Claim 25.)

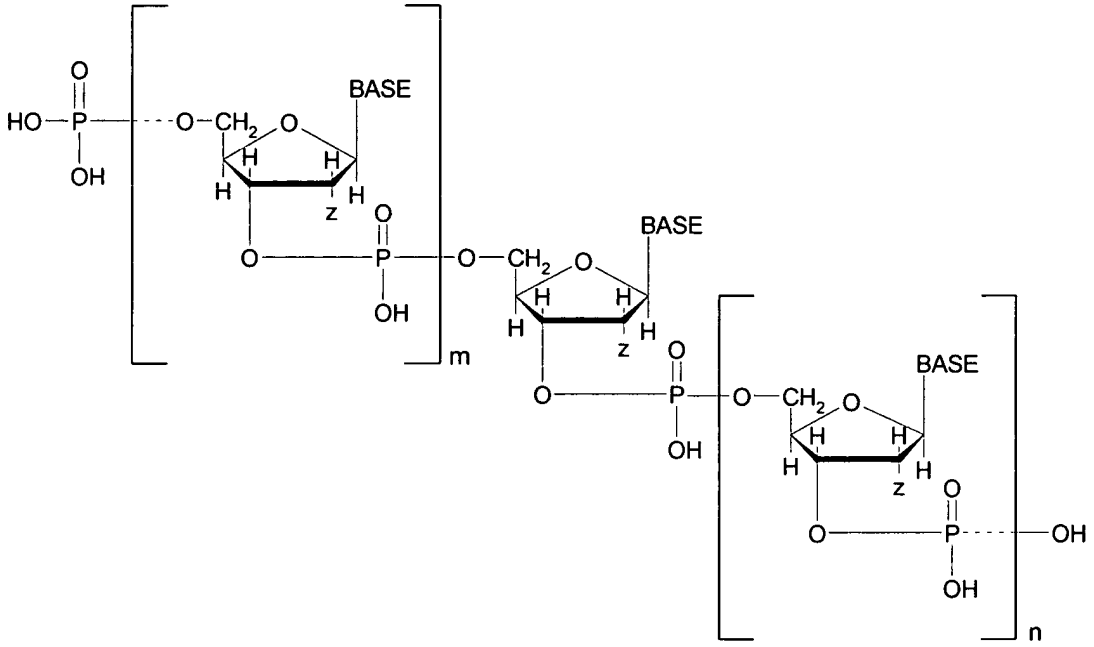
Sig can be biotin or iminobiotin. (Specification p. 10, ll. 11-12; Original Claim 92.) Sig can also be an electron dense component, such as ferritin. (Specification p. 97, ll. 5-7.) Alternatively, Sig can be a magnetic component, which includes magnetic oxides, such as ferric oxide. (Specification p. 96, l. 36-p. 97, l. 5.) Sig can also be an enzyme or an enzyme component, for example, alkaline phosphatase, acid phosphatase, β-

galactosidase, ribonuclease, glucose oxidase, or peroxidase. (Specification p. 96, ll. 30-34.) In another alternative, Sig can be a fluorescent component, such as fluorescein, rhodamine, or dansyl. (Specification p. 96, 34-36.) Further, Sig can be a hormone or hormone component, (Specification p. 102, ll. 1-3), a chemiluminescent component, (Specification p. 97, ll. 17-20), or an antigen (Specification p. 88, ll. 19-22; Original Claim 28). Also, Sig can be a metal-containing component, which can be catalytic. (Original Claim 28, 83, 174.) In yet another alternative, Sig can be a hapten, (Specification p. 97, ll. 10-13), or an antibody or an antibody component, (Original Claim 28), such that the antibody or hapten can be capable of complexing with the antibody of an antibody component (Original Claim 136). Sig can alternatively be complexed with a binding protein therefor, wherein the binding protein is conjugated to ferritin. (Specification p. 101, ll. 14-15.)

The oligo or polynucleotide can be terminally ligated or attached to a polypeptide. Sig can then be attached to the terminal nucleotide in the oligo or polynucleotide. (Original Claim 55, 167-8.) The sugar moiety of the terminal nucleotide can have hydrogens at either or both of the 2' and 3' positions thereof; alternatively, z of the terminal nucleotide can comprise a hydrogen at the 2' position thereto. (Specification p. 3, ll. 20-22.) Further, y and z of the terminal nucleotide can comprise a hydrogen at each of the 3' and 2' positions thereof, respectively. (*Id.*)

When the nucleotide is a deoxyribonucleotide, the oligo or polynucleotide can comprise at least one ribonucleotide. When the nucleotide is a ribonucleotide, the oligo or polynucleotide can comprise at least one deoxyribonucleotide.

When the nucleotide is a deoxyribonucleotide and Sig is attached to at least one of the phosphate moieties, the oligo or polynucleotide can also have the following structure:



wherein m and n are integers from about 0 to about 100,000.[2] (Specification p. 6, ll. 13-14.)

The present invention is also directed to compositions comprising the oligo or polynucleotides of the present inventive nucleic acid probes, (Original Claim 55, 167-168), a polypeptide capable of forming a complex with Sig, (Original Claim 55, 167-168), and a moiety, which is detectable when such complex is formed (Specification p. 8, l. 28-p. 9, l. 2).

The polypeptide of the present inventive compositions can be polylysine. (Original Claim 56.) Alternatively, the polypeptide can be avidin, strepavidin, or anti-Sig

[2] M and n are defined in the Specification at, for example, page 6, lines 13-14, and appear, for example, in claim 510. Not having any other meaning, these must be interpreted to be integers from about 0 to about 100,000.

immunoglobulin. (Specification p. 25, l. 36-p. 26, l. 35.) In another alternative, when Sig is a ligand, the polypeptide is an antibody thereto. (Specification p. 101, ll. 1-12.)

VI. ISSUES

There are three issues on appeal. First, whether claims 459-472 and 474-567 are unpatentable under 35 U.S.C. § 112, first paragraph, as lacking adequate written description. Second, whether claims 454-567 are unpatentable under 35 U.S.C. § 112, first paragraph, as being based on a non-enabling disclosure. Third, whether claims 454-567 are unpatentable under 35 U.S.C. § 103 over Halloran & Parker, *J. Immunol.*, 96(3): 373-78 (1966) **or** Miller et al., *Biochem.*, 20(7): 1874-80 (1981).

VII. GROUPING OF CLAIMS

Appellants submit that claims 454-567 do not stand or fall together. Each of claims 455, 457, 465-71, 474-81, 483, 485, 493-99, 474-79, 508-10, 512-13, 522-24, 526-28, 531-34, 536-37, 540, 542, 550-56, 559-62, 564-65, and 567 are drawn to specific embodiments that include an element that is not shown or suggested in the cited art. Therefore, appellants submit that each of the following groups of claims separately stand and fall together:

(1) Claims 454, 456, 458-64, 472-73, 482, 484, 486-492, 500-01, 506-07, 510 stand and fall together.

(2) Claims 511, 514-21, 525, 529-30, 535, 538-39, 541, 543-49, 557-58, 563, and 566-67 stand and fall together.

(3) Claims 455 and 483 stand and fall together.

(4) Claims 457 and 485 stand and fall together.

(5) Claims 465-68 and 493-96 stand and fall together.

(6) Claims 469 and 497 stand and fall together.

(7) Claims 470 and 498 stand and fall together.

(8) Claims 471 and 499 stand and fall together.

(9) Claims 474-79 and 502-05 stand and fall together.

(10) Claims 480-81 and 508-09 stand and fall together.

(11) Claims 512 and 540 stand and fall together.

(12) Claims 513 and 542 stand and fall together.

(13) Claims 522-24 and 550-53 stand and fall together.

(14) Claims 526 and 554 stand and fall together.

(15) Claims 527 and 555 stand and fall together.

(16) Claims 528 and 556 stand and fall together.

(17) Claims 531-34 and 559-62 stand and fall together.

(18) Claims 536-37 and 564-65 stand and fall together.

VIII. ARGUMENT

The present invention is directed to novel nucleic acid probes characterized by a Sig label covalently bound to the phosphate moiety PM, such that the oligo or polynucleotides are non-radioactively detectable. The present application contains a written description of these oligo or polynucleotides including broad disclosure of attachment of the Sig label to the phosphate moiety and examples of that attachment via the phosphate oxygen atom and also via the phosphate phosphorus atom.[3] One of skill in the art would undoubtedly appreciate from this disclosure that the inventors were in possession of all of the claimed subject matter at the time of filing. This is confirmed

[3] The only two types of atoms available are the phosphorus atom and the oxygen atom.

first by the Declaration of Dr. Dean L Engelhard and later by the Declaration of Dr. Cheryl H. Agris. Indeed, contrary to the Office positions, the original disclosure amply describes the specific chemical composition of the linkages between Sig and the PM, the specific identity of the labels and points of attachment of Sig to the phosphate atom of the PM, as well as, the compositions relating to the present inventive oligo or polynucleotides. As explained by Drs. Engelhardt and Agris, the chemistry for attaching numerous functional groups to nucleotides via the phosphate moiety was routine at the time of filing the application and would not have required undue experimentation.

The application also provides sufficient detail such that a person skilled in the relevant art would be able to make the claimed probes by covalently attaching or chemically linking a label, i.e., a Sig, to a phosphate moiety PM of a nucleotide and produce a modified oligo or polynucleotide for use as a nucleic acid probe without undue experimentation at the time of filing.

Finally, the claimed non-radioactively detectable nucleic acid probes are neither described in, nor suggested by, Halloran & Parker or Miller et al., for the reasons set forth in detail below.

A. The Present Application Contains a Sufficient Written Description Of The Inventive Oligo or Polynucleotides That One of Skill In the Art Would Know That the Inventors Were In Possession of the Claimed Subject Matter At the Time of Filing

1. Summary of the Recent Prosecution History

In the final Office Action dated July 18, 2000, claims 459-472 and 474-575 were rejected under 35 U.S.C. §112, first paragraph, as containing subject matter that was not adequately described in the specification in such a way as to reasonably convey to one of skill in the relevant art that the inventors, at the time of filing, were in possession

of the claimed invention.[4] According to the Office, support was not found for (1) the specific chemical composition of the linkages between Sig and the nucleotide (claims 459-463); (2) the specific identity of the labels and points of attachment of the Sig to internal phosphates (claims 464-472 and 482-569); or (3) compositions relating to any of limitations described above (claims 474-477 and 570-575). (Office Action – July 18, 2000, at 2.)

In traversing this rejection, appellants specifically addressed points (1)-(3). Initially, appellants pointed out at least nine instances in the original specification that recite the broad structure of the chemical linkages between Sig and the nucleotide. (*See, e.g.,* Amendment – January 18, 2001, at 21-22.) Appellants also have shown, in detail, the specific identity of labels (Sig) and points of attachment of the labels to internal phosphates. (*See, e.g.,* Amendment – January 18, 2001, at 23-31.) One of skill in the art, according to appellants and, more importantly, declarants (Drs. Engelhardt and Agris), a person actually skilled in the art, would find such description sufficiently detailed that one skilled in the art can reasonably conclude that the inventors had possession of the specific labels and points of attachment of the label to the phosphorus moiety, including internal phosphates. (*See Id.*; Agris Declaration ¶ 21-22.) Moreover, Dr. Agris detailed how she reached her conclusion that the description of the claimed embodiments encompassing attachment of Sig to the phosphorus or oxygen atoms of the phosphates meet the written description requirement. (*See* Amendment – January 18, 2001, at 31-34; Agris Declaration ¶ 20, 22-26.) Appellants also separately discussed these rejections in light of the composition claims. (*See* Amendment – January 18, 2001, at 34-36.)

[4] Although the Office has occasionally characterized this rejection as a "new matter" rejection under 35 U.S.C. § 132, this rejection was solely made under section 112.

In the Advisory Action, the Office responded that appellants' arguments, including the declaration, were not persuasive.[5] The Office rejected the facts presented by Dr. Agris in her declaration regarding what one of skill in the art would understand the specification to disclose. In addition, the Office found that the original specification and claims required that Sig be attached to the oxygen atom of the phosphate moiety.

2. The Specification Provides Sufficient Guidance Such that One Of Skill In The Art Would Understand That the Inventors Were In Possession Of The Claimed Invention At The Time Of Filing

It is clear to one of skill in the art that the inventors were in possession of the presently claimed invention when it was filed. The evidence demonstrates that the inventors intended to encompass within the scope of their original invention non-radioactively labeled nucleotides, particularly those nucleotides labeled at the phosphate moiety PM, formerly understood in the art to be unsuitable for labeling of nucleic acid hybridization probes. Also, the record includes declarations providing factual evidence of such written description that have not been fully addressed by the Office.

35 U.S.C. § 112, first paragraph, requires that an applicant provide "a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise and exact terms . . . ." To fulfill the written description requirement, a patent specification must describe the invention and do so in sufficient

[5] The Office parenthetically noted that portions of the specification were deemed not relevant and had been cancelled. Appellants' attorney explained during the Examiner Interview that the first fifty-two pages of the specification contained the patent disclosure of Ward et al. (U.S. Patent Application No. 255,223). Appellants' attorney also indicated that the Ward disclosure was initially deleted in the June 7, 1995 Preliminary Amendment to improve the readability of the present specification. After careful consideration and in light of the direction taken by the prosecution, Appellants' attorney decided to re-insert Ward's patent disclosure in the June 22, 2000 Second Supplemental Amendment. (*See* July 11, 2001 Communication at 5, 6.) Thus, everything relied on by appellants was included in the original disclosure and presently included in the Specification. All of this has no legal significance bearing on the issues in this case.

detail that one skilled in the art can clearly conclude that the inventor possessed the claimed invention at the time of filing. *Lockwood v. American Airlines, Inc.*, 107 F.3d 1565 (Fed. Cir. 1997); *see also Regents of the Univ. of Cal. v. Eli Lilly & Co.*, 119 F.3d 1559, 1569 (Fed. Cir. 1997).

"Satisfaction of the written description requirement *does not* require *in haec verba* antecedence in the originally filed application." *Staehelin v. Secher*, 24 USPQ2d 1513, 1519 (Bd. Pat. App. & Interf. 1992) (emphasis in original). Whether a specification complies with the written description requirement is a question of fact. *Vas-Cath Inc. v. Mahurkar*, 935 F.2d 1555, 1563 (Fed. Cir. 1991). Any declarations submitted by one of skill in the art must be considered as factual evidence. *See in re Alton*, 37 USPQ2d 1578, 1583 (Fed. Cir. 1996). "Precisely how close the description must come to comply with § 112 must be left to case-by-case development," *In re Smith*, 173 USPQ 679, 683 (CCPA 1972), to be determined on the totality of the record. *See In re Oetiker*, 977 F.2d 1443, 1445 (Fed. Cir. 1992).

Appellants point out that, with respect to claims 568-575, the rejection has been rendered moot in light of the cancellation of these claims. Thus, the remarks below are directed to the written description rejection insofar as it applies to the remaining claims 459-472 and 474-567. Appellants also note that the written description requirement is not applied to claims 454-58 and 473, although these claims include attachment of Sig to the phosphate moiety, which includes attachment of Sig to both the phosphorus atom and the oxygen atom of the phosphate moiety.

In the Advisory Action, the Office has misconstrued the standard for written description. The Office stated:

> In mixing and matching portions of the specification (some of which applicants felt were not relevant and has cancelled from the specification)

> the Declaration fails to explain where the specification indicated that these portions must necessarily be combined in the declarant proposes
>
> . . .
>
> The Declaration does not explain how base linkages make phosphate linkages necessarily inherent.

(Advisory Action – May 30, 2001, at 3.)

First, there is no inherency requirement in the test for written description. As noted above, the specification need only convey to one of skill in the art that the inventors were in possession of the claimed subject matter. This record clearly shows the following facts, which the Office does not dispute.

- The original specification clearly describes all of the elements of the rejected claims (although not all the specifically claimed Sig—PM combinations, *in haec verba*).
- The original claims generically embraced the subject matter sought to be patented.
- The original disclosure expressly states that the inventors considered their invention to embrace linkages of all Sig labels to the phosphate moiety PM on the nucleotide.
- In fact, one of skill in the art concluded, based on the original disclosure, that attaching the Sig label to the phosphate moiety via all known means was within the scope of what the inventors possessed at the time of filing.

a. The Record Plainly Demonstrates that the Inventors Were in Possession of the Presently Claimed Invention as of the Filing Date

The original disclosure provides for Sig to be attached directly or indirectly to the phosphate moiety PM nucleotide. This unrebutted fact has been made abundantly clear in the record. (*See* Amendment – January 18, 2001, at 23-31; Agris Declaration ¶ 21-26.) Attachment of Sig to the oxygen atom of PM is set forth in the description of the invention, while attachment of Sig to the phosphate atom of PM is set forth in Example V of the invention. (*Id.*; *see also* Specification at 57.) Specifically in Example V, both biotin and polybiotinylated poly-L-lysine were coupled to oligodeoxyribonucleotides using a carbodiimide coupling procedure described in Halloran and Parker. The Office even admits that "Halloran discloses the attachment of a specific signal moiety, a protein, to the phosphorus atom of the phosphate moiety using a specific linker, a -C-$(CH_2)_4$-N- chain." (Final Office Action – July 18, 2000 at 3.) Further, attachment of Sig to various positions on the sugar moiety via a phosphate linkage have been described previously in the prosecution. (*See, e.g.*, Amendment – January 18, 2001, at 23-31; Agris Declaration ¶ 21-26.)

It is important to take into consideration that Example V must be read in conjunction with Examples I-III, which support the preparation of Example V. Example I demonstrates the preparation of the activated ester of biotin. Example II supports the preparation of the amide form of biotin. Finally, Example III supports the preparation of polybiotinylated poly-L-lysine. All of these compounds were used in Example V, as shown in the following figure.

Relationship of Examples I-III to Example V
Pages 55-57

Example I	Preparation of Activated Ester of Biotin (Biotinyl-N-hydroxysuccinimide ester (BNHS)
↓	
Example III	(Preparation of Polybiotinylated poly-L-lysine)
↓	
Example V	(Coupling of Polybiotinylated poly-L-lysine to Oligoribonucleotides
Example V	(Coupling of Biotin to Oligoribonucleotides)
↑	
Example II	(Prepartion of Amide Form of Biotin) (Biotinyl-1,6-diaminohexane amide)

Although disclosure of attachment of all Sig labels to all disruptive and semi-disruptive sites of the PM on the nucleotide are not specifically set forth in the same way in the original disclosure, they are all adequately described. Taking into account what one of skill in the art would appreciate from the original disclosure, particularly, that the inventors were in possession of the broad invention, i.e., binding Sig labels at disruptive and semi-disruptive sites of the phosphate moiety PM, and that obviously the inventors were in possession of all the disclosed possibilities for Sig labels and binding such Sig labels to the PM, the question before this board is whether they should be denied specific claimed combinations on the ground of lack of written description. The answer is no.

The Office points to nothing more than the fact that every claimed specific combination is not disclosed literally. That test would place an unreasonable disclosure burden on applicants, especially where there are numerous permutations, and is certainly a burden not required by 35 U.S.C. § 112, first paragraph.

In setting forth the written description rejection, the Office relied on the fact that the originally filed claims required that Sig be attached to the oxygen atom of PM. Relying solely on the original claims, in contradiction of the specification and declarations of experts, the Office concludes that the subject matter was not in the possession of the inventors at the time the application was filed. The only apparent basis for that position is that anything not specifically laid out in the original claims cannot subsequently be claimed. This position has been consistently rejected as a condition of patentability.

b. The Office Has Not Adequately Addressed the Factual Issues Set Forth in the Expert Declarations

The Examiner has substituted his own judgement for that of the person skilled in the art. It is apparent from the Advisory Action that the Office has not given the proper weight to the facts presented in the Engelhardt and Agris Declarations, of what one of skill in the art would understand from the original disclosure. In essence, all that has been stated by the Office is that the Examiner has a different opinion than that of the experts.

The Office has not fully considered the totality of the record, which is required. Having failed to articulate adequate reasons in rebuttal to the Agris Declaration, the Office clearly did not discharge its burden in this regard. *See* MPEP §§ 716, 716.01. The Office also has not provided specific reasons why one of skill in the art would not have understood that the inventors were in possession of the claimed invention at the time the present application was filed, other than to state that the embodiments in the rejected claims are not inherent in the original disclosure.

As such, maintenance of the written description rejection is improper and it should be withdrawn.

c. The Invention is Intended to Encompass Nucleic Acid Hybridization Probes wherein the Sig Label is Attached to the Modified Nucleotide via the Phosphate Moiety

The original disclosure details how the present invention is directed to attachment of non-radioactive labels in what previously had been considered unsuitable positions, for example, the phosphate moiety. In U.S. Patent Nos. 4,711,955, 5,328,824, 5,499,767, 5,476,928 issued to Ward et al., Ward detailed very specific attachment positions of a non-radioactive label to the base moiety of a nucleotide. According to Ward, non-radioactive labels could only be attached or placed on ring positions of the base in a manner that did not interfere with hydrogen bonding of the bases. By disruptive, it was thought that the non-radioactive label could not be attached at certain points on the nucleotide because it would "disrupt" the capability of the labeled nucleotide to function as an oligo or polynucleotide for use as a hybridization probe.

Headlong against the teachings in the art, and particularly Ward, the present inventors discovered that non-radioactive labels could be incorporated into nucleic acid probes via the PM, base or sugar of the nucleotide. It is this discovery relative to the PM that is set forth in the rejected claims of the present application. Accordingly, the inventors of the present invention had possession of the invention encompassing attachment of the label (Sig) to the new attachment points of the nucleotide.

Furthermore, it is clear from the Declaration of Dr. Engelhardt[6] that the inventors intended to encompass, in contrast to the Ward invention, "disruptive" or "semi-disruptive" positions in the oligo or polynucleotide of the probe. (Engelhard Declaration ¶ 10A; *see also* Agris Declaration Ex. 6 ¶ 10A.) "It is very clear in my opinion that the specification discloses that the embodiments of Sig are to be applied – without limitation – in the disruptive and semi-disruptive positions of all three moieties recited in the independent claims, i.e., the base, sugar and phosphate moieties." (*Id.*)

The only point of contention here appears to be that some of the specific Sig labels and PM combinations claimed are not ____ disclosed. It is respectfully submitted that the record shows that each element of these combinations was embraced by what applicants considered their invention. Furthermore, each element of these combinations was disclosed and selection of the claimed combinations would have been apparent to one skilled in the art. There is nothing in the original disclosure that was pointed to, nor is there anything that can be pointed to, suggesting that the inventors did not consider all these combinations to be part of their invention.

B. The Subject Matter Is Described In the Specification Such That It Enables One Skilled In the Art To Make and Use the Present Invention

1. Summary of the Recent Prosecution History

In the final Office Action dated July 18, 2000, claims 454-575 were rejected under 35 U.S.C. §112, first paragraph, as containing subject matter that is not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention. According to

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[6] The Engelhardt Declaration was originally filed November 24, 1997, with an Amendment Under 37 C.F.R. § 1.116 In Response to June 25, 1997 Office Action and was subsequently included as Exhibit 6 to the Agris Declaration in the July 18, 2001 Amendment.

the Office, the claims are drawn to a broad generic Sig and any chemical linkages relating thereto, while the disclosure only describes attachment of a specific Sig using a specific linker. (*See* Office Action – July 18, 2000 at 3.) Accordig to the Office, the claims are broader than the disclosure and, therefore, the scope of enablement is not commensurate with the scope of the claims. In addition, the Office concluded without support that it would require undue experimentation to determine which of the various Sig labels would produce a functional oligo or polynucleotide. (*Id.* at 3-4.)

In traversing the enablement rejection, appellants demonstrated that, armed with the disclosure of the present specification, one of skill in the art would be able to make and use the claimed invention. Appellants pointed out that Halloran & Parker teach that the chemistry necessary for attachment of protein to the phosphate moiety of a nucleotide. The original disclosure recited various Sig labels that could be attached to the PM moiety of the nucleotide by means known in the art. (*See* Amendment – January 18, 2001, at 37; Agris Declaration ¶ 32.) One such disclosure is found in Example V, and related Examples I-III, where appellants teach a method for attaching particular embodiments of Sig, biotin or biotinylated poly-L-lysine, to the phosphorus atom or oxygen atom of the phosphate moiety. (*Id.*) Appellants further demonstrated through the Engelhardt Declaration that the chemistry and reactions for attaching substituents to the oxygen or phosphorus atoms in a nucleotidyl phosphate or phosphoric acid moieties were known in the art at the time the initial application was filed in June 1982. (*Id.*; *see also* Agris declaration, Exh. 6.)

In response to the contention of the Office that there is some question as to which of the Sig embodiments will be operative, appellants turned to Dr. Agris for explanation of what one of skill in the art would have known at the time the present application was filed. Dr. Agris indicated that, "[a]lthough Armstrong et al. does disclose

that some modified nucleotides are better than others in terms of binding to RNA polymerase, a person skilled in the art would expect that some routine testing or refinement is necessary" to reach all the different types of Sig labels. (Agris Declaration ¶ 33; *see also* Amendment – January 18, 2001, at 38-39.)

In the Advisory Action, the Office indicated that the arguments submitted in support of enablement were not considered persuasive. Again, the Office focused on the lack of disclosure for attachment of the Sig labels to the phosphate moiety PM. (*See* Advisory Action – May 30, 2001, at 5.) According to the Office, the disclosure in Example V cannot be enabling because appellants have argued that the Halloran & Parker paper does not render the present invention obvious. Again, the Office misses the point, Halloran & Parker teach methods for conjugating protein to the PM on a nucleotide, not a nucleic acid hybridization probe.

Also, in the Examiner Interview, the Office reiterated its position that the disclosure did not enable the attachment of the various chemical linkages disclosed in the specification to the phosphate moiety. (*See* Communication – July 11, 2001, at 7.) However, the Office did not identify which if any of the chemical linkages or reaction conditions posed an enablement synthetic hurdle which are beyond the level of the ordinarily skilled organic chemist. Again, Appellants responded by referring to the Engelhardt Declaration, pointing out almost a dozen papers that show modifications (but no non-radioactive detectable labels) to the oxygen or to the phosphorus atom in the phosphate moiety. *Id.*

2. One of Skill in the Art Would be Able to Make and Use the Claimed Non-Radioactively Detectable Nucleic Acid Probes without Undue Experimentation

35 U.S.C. § 112, first paragraph, requires that an applicant provide an enabling disclosure (emphasis added):

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise and exact terms as *to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same* . . .

Enablement is a question of law based on underlying factual inquiries. *See National Recovery Techs., Inc. v. Magnetic Separation Sys., Inc.*, 166 F.3d 1190 (Fed. Cir. 1999). The standard for determining if a specification is enabling requires that a person skilled in the relevant art be able to make and use the invention without undue experimentation at the time of filing. *See United States v. Telectronics, Inc.*, 857 F.2d 778 (Fed. Cir. 1988), *cert. denied*, 490 U.S. 1046 (1989). The specification need not disclose what is known in the art. *See In re Buchner*, 929 F.2d 660, 661 (Fed. Cir. 1991). Further, the scope of the claims must bear a reasonable correlation to the scope of enablement provided by the specification. *See in re Vaeck*, 947 F.2d 488 (Fed. Cir. 1991) (holding that claim not enabled because there was no reasonable correlation between the narrow disclosure in the specification and the broad scope of protection sought.); *In re Goodman*, 11 F.3d 1046 (Fed. Cir. 1993) (holding that the specification must teach those of skill in the art how to make and used the invention as broadly as it is claimed.); *see also PPG Indus., Inc. v. Guardian Indus. Corp.*, 75 F.3d 1558 (Fed. Cir. 1996) ("In unpredictable art areas, this court has refused to find broad generic claims enabled by specifications that demonstrate the enablement of only one or a few

embodiments and do not demonstrate with reasonable specificity how to make and use other potential embodiments across the full scope of the claim.").

Exactly what constitutes undue experimentation is decided on the facts of each particular case, see *Ex parte Forman*, 230 U.S.P.Q. 546, 547 (Bd. Pat. App. & Interf. 1986), with the *Wands* factors serving as a guide to ascertain what constitutes undue experimentation. *In re Wands*, 858 F.2d 731, 737 (Fed. Cir. 1988). According to *Wands*, these factors are (1) the quantity of experimentation necessary; (2) the amount of direction or guidance presented; (3) the presence or absence of working examples; (4) the nature of the invention; (5) the state of the prior art; (6) the relative skill of those in the art; (7) the predictability or unpredictability of the art; and (8) the breadth of the claims. *Id.* Whether claims are sufficiently enabled by the specification is determined as of the filing date of the patent application. *See United States Steel Corp. v. Phillips Petroleum Co.*, 865 F.2d 1247 (Fed. Cir. 1989).

a. The Original Disclosure Teaches How to Attach a Non-Radioactive Label to an Oligo or Polynucleotide and How to Use the Resulting Probe in the Detection of Nucleic Acids

The scope of the claims bears a correlation to the scope of enablement provided by the specification. The claims are directed to oligo or polynucleotides that have at least one modified nucleotide in which a label (Sig) is covalently attached to the nucleotide of the oligo of polynucleotide either directly or via a chemical linkage. The claimed oligo or polynucleotides are nucleic acid hybridization probes.

The Office has never identified, nor can it identify, any Sig and corresponding chemical linkages or reaction conditions that would pose an enablement synthetic hurdle beyond the level of one of ordinarily skill in the art. As pointed out throughout the

prosecution of the present application, Halloran & Parker exemplifies conjugation and coupling of poly-L-lysine to a nucleotide. Attachment of Sig labels using the coupling methods of Halloran & Parker is set forth in the specification in Example V, and related Examples I-III. Example V exemplifies attachment of two separate labels, biotin and polybiotinylated poly-L-lysine, to oligoribonucleotides at both the oxygen and phosphate atoms of PM. (*See* Specification p. 57, ll. 1-15.)

Furthermore, as explained in the Engelhardt Declaration, the chemistry and reactions for attaching substituents to the oxygen or phosphorus atoms of the PM in the claimed oligo or polynucleotide were known in the art at the time the initial application was filed in June of 1982. Thus, the coupling reactions for attaching the Sig label to the PM of the nucleotide were well within the routine skill of one in the art and would not require undue experimentation. Furthermore, one of skill in the art would have found the known chemistry and reactions entirely predictable. (*See* Amendment – January 18, 2001, at 37; Agris Declaration Ex. 6.)

b. The So-Called 112/103 Crunch Does Not Exist in this Case

In crafting the non-enablement argument, the Office alleges that appellants are in a 112/103 crunch because the same reference is at issue in both the enablement and obviousness context. According to the Office, the disclosure cannot be considered enabling without rendering the present invention obvious. This is just incorrect reasoning. Appellants are simply using the Halloran & Parker reference to show that a skilled artisan would have known at the time of filing the various methods for covalent or chemical linkage of a Sig label to the PM of a nucleotide or an oligo or polynucleotide. As is well accepted, the specification need not disclose what is known in the art. It is

only when this knowledge is combined with appellants own disclosure relating to the use of a non-radioactive label bound to the PM moiety of the claimed nucleic acid probes that one of skill in the art would have been able to make and use the present inventive oligo and polynucleotide probes.

c. The Possible Existence of Inoperative Labels Does Not Alter Enablement of the Claims

The Office incorrectly construes the standard relating to inoperative subject matter. According to the Office, the potential for inoperative embodiments renders the claims non-enabled. This is an incorrect statement. The correct standard is whether a person of ordinary skill in the art could determine which embodiments would be inoperative or operative with expenditure of no more effort than is normally required in the art. *Atlas Powder Co. v. E.I. du Pont de Nemours & Co.*, 750 F.2d 1569, 1577 (Fed. Cir. 1984). As stated by Dr. Agris is her declaration, one of skill in the art would be able to determine with "routine testing or refinement " which of the Sig labels was operative and, thus, encompassed by the claims. (Agris Declaration ¶ 33.)

C. The Present Invention is Not Obvious in Light of the Prior Art

1. Summary of the Relevant Prosecution History

In the final Office Action dated July 18, 2000, claims 454-575 were rejected under 35 U.S.C. § 103 as unpatentable over the Halloran & Parker paper **or** the Miller at al. paper.[7] (*See* Office Action – July 18, 2000 at 4.) According to the Office, Halloran & Parker and Miller et al. teach attachment of specific molecules to nucleic acids. As

[7] Because the Office withdrew the obviousness rejection under 35 U.S.C. § 103(a) over U.S. Patent No. 4,378,458 (Gohlke et al.) in view of Sodja et al., *Nucleic Acids Res.*, 5: 385-401 (1978) set forth in paragraph 5 of the Final Office Action, this rejection will not be discussed herein. (*See* Communication – July 11, 2001, at 9.)

such, the Office contends it would have been *prima facia* obvious to substitute any linker or Sig label in the methods of these references to arrive at the present invention with a reasonable expectation of success. (*Id.*) The Office also attempts to fashion a 112/103 crunch wherein appellants cannot use the prior art to buttress their enablement arguments without conceding the obviousness of the present invention. (*Id.* at 5.)

In response, appellants argued that one of skill in the art would not arrive at the present invention absent the teachings in the present specification. Appellants presented evidence from Dr. Agris that the Miller et al. and Halloran & Parker references were obviously used as guidelines in formulating the oligo or polynucleotides of the present inventive hybridization probes. (*See* Agris Declaration ¶ 39; Amendment – January 18, 2001, at 45-46.) According to Dr. Agris, however, there was no suggestion that these procedures could or should be used to obtain, nor would one of ordinary skill in the art have a reasonable expectation of success in obtaining the nucleic acid hybridization probes without the teaching in the present specification. (*Id.*) For that matter there can be no motivation to make what is not disclosed. The Office does not point to any teaching in the art, nor is there any such teaching that binding a non-radioactive Sig label to the PM of a nucleotide could produce a useful hybridization probe. To the contrary, at the time the invention was made, it was the view in the art that such binding would disrupt the oligo or polynucleotide and, therefore, a useful hybridization probe could not be made binding the Sig label to the PM moiety.

In the Advisory Action, the Office found the arguments submitted unpersuasive. Particularly, the Office alleged that the claims were directed to a product that is not limited to a specific use and, therefore, the prior art inherently teaches the present invention. (*See* Advisory Action – May 30, 2001, at 6.)

In response, Appellants discussed in the Examiner Interview how neither Halloran & Parker nor Miller et al. disclosed the instantly claimed non-radioactively labeled detectable nucleic acid hybridization probes. Appellants explained that the moieties taught in Halloran & Parker were not non-radioactively detectable nucleic acid hybridization probes because no label in the form of a chemical modification was ever made to the protein or nucleic acid, no hybridization was ever undertaken, and no signal was ever generated. (*See* Communication – July 11, 2001, at 7.) Regarding the Miller et al. reference, appellants explained that, unlike the non-radioactively detectable moieties of the present invention, the moieties of Miller et al. were radioactively labeled with tritium (^{3}H). *Id.*

A claim is invalid if the differences between the subject matter the applicant seeks to patent and the prior art are such that one of ordinary skill in the art to which the subject matter pertains would find the subject matter as a whole obvious at the time of making the invention. 35 U.S.C. § 103(a). This inquiry is a question of law based on factual inquiries. In *Graham v. John Deere Co.*, 383 U.S. 1 (1966), the Supreme Court set forth the criteria to determine the obviousness or unobviousness of a claimed invention. This includes a factual finding respecting: (1) the scope and content of the prior art; (2) the level of ordinary skill in the art; (3) the differences between the claimed invention and the prior art; and (4) secondary considerations, or objective indicia of nonobviousness, such as a long-felt need for the claimed invention, commercial success of the invention, and failure of others to achieve the invention. *Id.* at 17-18.

For a *prima facia* case of obviousness, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skin the art, to modify the reference or to combine reference teachings, there must be a reasonable expectation of success, and, the prior art

reference (or references when combined) must teach or suggest all the claim limitations. The teaching, suggestion, or motivation to combine the references, and the reasonable expectation of success, must be found either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, and not based or applicant's disclosure. *In re Fine*, 837 F.2d 1071, 1074 (Fed. Cir. 1988).

2. Neither Halloran & Parker nor Miller et al. Disclose the Claimed Invention

The oligo and polynucleotides[8] defined by the rejected claims 454-567 are non-radioactive nucleic acid hybridization probes characterized, *inter alia*, by at least one modified nucleotide having a "Sig" label that is covalently attached, directly or via a chemical linkage, to the phosphate moiety "PM"of the modified nucleotide. The Sig label is capable of non-radioactive detection when attached to the PM or when the modified nucleotide is incorporated into the oligo or polynucleotide.

A hybridization probe is understood in the art to be an oligo or polynucleotide that is complementary to a nucleic acid sequence under investigation. Hybridization of the probe to the nucleotide sequence of interest allows that sequence to be detected.

The Office refused to enter all the amendments presented in the Rule 1.116 Amendment in the Advisory Action of May 30, 2001. Some of those amendments expressly state implicit features of a hybridization probe, e.g., that the oligo- or polynucleotide is complementary to a nucleic acid of interest, or clarify related features of the claims, e.g., that Sig is a non-radioactive label that can be detected when hybridized to the complementary nucleic acid of interest or portion thereof. Notably these amendments were not among those objected to in the Advisory Action and,

[8] More specifically, claims 454 to 510 are directed to deoxyribonucleotides; claims 511 to 567 are directed to ribonucleotides.

consistent therewith, at the Interview of July 11, 2001, the Examiner agreed that, properly interpreted, at least some of the rejected claims, e.g., claim 454, are directed to hybridization probes. This interpretation is also consistent with the fact that the rejections are under 35 U.S.C. § 103(a) rather than 35 U.S.C. § 102.

Submitted herewith is a Second Amendment under Rule 1.116 in accordance with MPEP § 1207. The amendments proposed for entry are limited to expressing what are respectfully submitted to be inherent or implicit features of the existing claims or clarifications. It is believed that these amendments put the claims in condition for allowance or at least obviate some issues for Appeal.

a. It Is Undisputed That Halloran & Parker Does Not Disclose Or Suggest The Hybridization Probe Of The Properly Interpreted Claims

According to the Office, (Office Action, May 30, 2001, at 6), the prior art renders the claimed hybridization probes obvious under 35 U.S.C. § 103(a) based on the following four points.

(1) The Engelhardt Declaration at pages 11 and 12 states that the chemistry and reactions for attaching substituents to the oxygen or phosphorus atom in a nucleotidyl phosphate or phosphoric acid moiety were already known in the art at the time the initial application was filed in June 1982.[9]

(2) The Sig label "Biotin" was known in the prior art as a tightly bound non-polypeptide structure required for the activity of an enzyme or other

9 This, of course, is inconsistent with the non-enablement rejections, but has little relevance to the question of whether binding Sig labels to disruptive or semi-disruptive sites on the PM moiety of a nucleotide would produce a hybridization probe.

protein, i.e., a prosthetic group, and other proteins were known as Sig labels due to the fact that antibodies can specifically detect them.

(3) Halloran teaches the attachment of proteins to phosphate groups.

(4) The rejected claims are product claims and not limited to any use.

Points 1 and 3 are not disputed.

As to dispute point 2, it is submitted that non-radioactive label bound to the PM of a nucleotide for use as a hybridization probe were not known in the art.

As to disputed point 4, it is submitted that, properly interpreted, the rejected claims are at least *de facto* "use" claims because they are limited to hybridization probes that, properly interpreted, require that use because the claims recite that the Sig label is capable of non-radioactive detection *when* attached to the PM or *when* the modified nucleotide is incorporated into the oligo or polynucleotide.

Halloran & Parker is directed to preparation of nucleotide protein conjugates in which a protein is covalently linked to a nucleotide with a carbodiimide coupling agent. This field of endeavor is different from that of the claimed invention. Halloran & Parker report that they selected carbodiimide coupling agents because they were known to promote three possible coupling reactions of nucleotides with proteins in the presence of carbodiimindes. Figure 1 illustrates the three coupling reactions. The first coupling reaction employs a terminal phosphate oxygen to atom form a phosphate diester bond with a seryl or threonyl protein residue. The second coupling reaction employs an epsilon-amino group on the protein to form an N-P bond with the terminal phosphate

group phosphorous atom. The third coupling reaction employs the 2' hydroxyl group of a terminal sugar to form and ester with a carboxyl group on a protein. What these teachings do show is that the chemistry of linking various moieties to phosphate groups was well understood at the time the instant application was filed.

The protein is intended to provide an antigenic site on the nucleotide while preserving its structural integrity and it is stated that the conjugates of proteins with mononucleotides, oligonucleotides and DNA elicit antibodies with nucleotide specificity.

It is indisputable that Halloran & Parker does not disclose or suggest the hybridization probe of the properly interpreted rejected claims.

What Halloran & Parker disclose must be considered in the context of all relevant prior art. For example, Halloran & Parker published their work in 1966, but it was not until approximately fifteen years later that Ward made his breakthrough discovery regarding the non-radioactive, non-disruptive base labeling of nucleic acids. According to one of the present inventors, Dr. Engelhardt, "[p]rior to 1981, nucleic acids were conventionally labeled with radioactive isotopes, most notably ^{32}P. With Dr. Ward's discovery, the world turned en masse to non-radioactive labeling of nucleic acids . . . ." (Engelhardt Declaration ¶ 8A; *see also* Agris Declaration Ex. 6, ¶ 8A.) As discussed above, Ward concluded that such non-radioactive probes required binding of a label to a limited number of "non-disruptive" binding sites on the base. *See*, *e.g.*, U.S. Patent No. 4,711,955; U.S. Patent No. 5,328,824; U.S. Patent No. 5,499,767; U.S. Patent No. 5,476,928. "[The inventors] subsequent and unexpected discovery that culminated in the filing of the first application in the family in 1982 flew headlong against Ward because the positions for labeling the nucleic acid now involved the so-called 'disruptive' and 'semi-disruptive' positions in the base." (*Id.* ¶ 9A.) Thus, Ward teaches away from attaching a label anywhere on a nucleotide other that the non-disruptive base positions

on a nucleotide for a hybridization probe, including binding a label to the phosphorus moiety.

The Declaration of Cheryl H. Agris, executed January, 17, 2001, (¶ 34-39), establishes that to a person of at least ordinary skill in the art, Halloran & Parker does not disclose or suggest that the final oligo or polynucleotide claimed, i.e., a non-radioactive hybridization probe, could be obtained.

Clearly, it is only with the benefit of hindsight knowledge of the present invention that the Office can conclude it would have been obvious to make a non-radioactive hybridization probe from the teaching of Halloran & Parker

It must also be taken into account that Halloran & Parker is cited in Example V, and related Examples I-III, of the subject patent application, (Specification p. 57), as a method for making a non-radioactive hybridization probe using carbodiimide to couple two separate non-radioactive labels –biotin and polybiotinylated poly-L-lysine – to oligoribonucleotides. As explained above, appellants prepared the activated ester of biotin in Example I, which they then used to prepare polybiotinylated poly-L-lysine in Example III. The later was then used in the coupling procedure of Example V. In Example II, appellants prepared the amide form of biotin, which was also used in the coupling procedure of Example V. In contrast to the claimed invention, Halloran & Parker did not teach or suggest that a non-radioactive label could be incorporated into a a nucleic acid hybridization probe. Nor did Halloran & Parker disclose that a non-radioactive label could be detectable so incorporated or when hybridized with a complementary nucleic acid of interest.

b. Halloran & Park Does Not Disclose, Much Less Suggest, Many of the Dependent Claim Limitations

The oligo and polydeoxyribonucleotides defined by the rejected claims 455-81 and 483-509 are non-radioactive nucleic acid hybridization probes characterized, *inter alia*, by at least one modified nucleotide having a "Sig" label that is covalently attached to a phosphate group, directly or via a chemical linkage, at disruptive or semi-disruptive positions of the modified nucleotide. Dependent claims 511-35 and 539-67 are directed to ribonucleotides. The Sig label is capable of non-radioactive detection when attached to the SM or when the modified nucleotide is incorporated into the oligo or polydeoxyribonucleotide.

The dependent claims are discussed below. These claims contain additional features neither disclosed nor suggested by Halloran & Parker. Furthermore, dependent claims 511-35 and 539-67 are directed to oligo or polyribonucleotide probes, which are not even suggested, much less taught in Halloran & Parker. Therefore, the rejection of all the following claims under 35 USC § 103(a) are clearly improper and should be reversed.

Claims 455, 483, 512, and 540 recite that the Sig label is or renders the nucleotide self-signaling, self-indicating or self-detecting. Halloran & Parker teach nothing about polydeoxyribonucleotides with self-signaling, self-indicating or self-detecting Sig labels. This embodiment does not, for example, require antibodies, and thus is advantageous because it can be detected directly.

Claims 457, 485, 513, and 542 recite that the Sig label is attached to the PM via the phosphate oxygen. The only conjugate made by Halloran & Parker is attached via the phosphate phosphorous atom.

Claims 465-68, 493-96, 522-24, and 550-53 recite electron dense Sig labels such as ferritin, ferritin conjugated to a protein, magnetic oxide and ferric oxide. Halloran & Parker teach nothing about electron dense Sig labels, or any of the recited species thereof. These self-signaling, indicating, or detecting Sig labels are advantageous to the hybridization probe and is not disclosed or suggested by the prior art.

Claims 469, 497, 526, and 554 recite that the Sig label is an enzyme selected from a specific group. Halloran & Parker does not disclose or suggest any enzyme linked to a nucleotide.

Claims 470, 498, 527, and 555 recite that the Sig label comprises a metal containing component that is catalytic. Halloran & Parker does not disclose or suggest a metal containing component that is catalytic.

Claims 471, 499, 528, and 556 recites that the Sig label comprises a selected fluorescent component. Halloran & Parker does not disclose or suggest any fluorescent component.

Claims 474-79, 502-05, 531-34, and 559-62 recite a composition including the oligo or polydeoxyribonucleotides of claim 454 and further including a polypeptide capable of forming a complex with a Sig label.

Claims 480-81 and 508-09 recite embodiments where the oligo or polydeoxyribonucleotide includes a terminal ribonucleotide. Halloran & Parker neither disclose nor suggest such a terminal ribonucleotide.

Claims 536-37 and 564-65 recite embodiments where the oligo or polyribonucleotide includes a terminal ribonucleotide with a hydrogen at either the 2' or 3' positions, or both. Halloran & Parker neither disclose nor suggest such a terminal ribonucleotide.

The dependent claims described in the preceding paragraphs are directed to features of the claimed hybridization probes. Halloran & Parker is completely silent on such probes and, therefore, could not motivate such modifications. Thus these claims are directed to features of the claimed hybridization probes that could not be obvious from Halloran & Parker because there can be no motivation to make something that is not even disclosed in the art relied on to support a rejection.

c. Miller et al. Does Not Teach Nor Suggest Non-Radioactively Labeled Oligo or Polynucleotides For Use as Nucleic Acid Hybridization Probes.

As discussed above, the rejected claims are limited to hybridization probes that require that the Sig label is capable of non-radioactive detection *when* attached to the PM or *when* the modified nucleotide is incorporated into the oligo or polynucleotide.

Miller et al. is directed to nonionic oligonucleotide analogues that have an isoteric 3'-5'-linked methylphosphonate group that replaces the normal phosphodiester linkage of nucleic acids. Again, this field of endeavor is different from that of the claimed invention. Miller et al. uses radioactive labels (^{3}H) for their probes in all the nucleic acid hybridizations. What these teachings show is that utilization of radioactive probes was the standard prior to Ward and the present invention.

It is indisputable that Miller et al. does not disclose or suggest the use of non-radioactive hybridization probes of the properly interpreted rejected claims.

IX. CONCLUSION

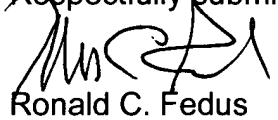
The present application contains a written description such that one of skill in the art would undoubtedly appreciate from this disclosure that the inventors were in

possession at the time of filing of the claimed non-radioactively detectable oligo or polynucleotides for use as nucleic acid probes. The application also provides sufficient detail such that a person skilled in the relevant art would be able to make the claimed probes by covalently attaching or chemically linking a label, i.e., a Sig, to the PM moiety of a nucleotide and produce a functional oligo or polynucleotide for use as a nucleic acid probe without undue experimentation at the time of filing. Moreover, the present non-radioactively detectable nucleic acid probes are neither described in, nor suggested by, Halloran & Parker or Miller et al. Accordingly, reversal of the Examiner's rejections is respectfully requested.

No extension request or fee is believed due in connection with this filing, a Request For An Extension Of Time (5 Months) and authorization for the fee therefor having been filed herewith. In the event that any fee or fees are due, The Patent and Trademark Office is hereby authorized to charge the amount of any such fee(s) to Deposit Account No. 05-1135, or to credit any overpayment thereto.

If a telephone conversation would further the prosecution of the present application, Applicants' undersigned attorney request that he be contacted at the number provided below.

Respectfully submitted,



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Enz-5(D6)(C2)

APPENDIX A
Amended Claims in Appeal

454. An oligo- or polydeoxynucleotide that is complementary to a nucleic acid of interest, or a portion thereof, comprising at least one modified nucleotide having the formula

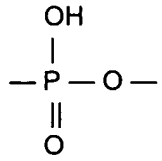
Sig—PM—SM—BASE

wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, said PM being attached to SM, said BASE being attached to SM, and Sig being covalently attached to PM directly or through a chemical linkage, said Sig being a moiety capable of non-radioactive detection when attached to PM, when said nucleotide is incorporated into said oligo- or polydeoxyribonucleotide, or when said oligo- or polydeoxyribonucleotide is hybridized to said complementary nucleic acid of interest, or a portion thereof.

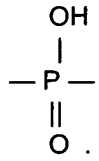
455. The oligo- or polydeoxyribonucleotide of claim 454, wherein said Sig is or renders the nucleotide self-signaling or self-indicating or self-detecting.

456. The oligo- or polydeoxyribonucleotide of claim 454, wherein said Sig moiety comprises at least three carbon atoms.

457. The oligo- or polydeoxyribonucleotide of claim 454, wherein said covalent attachment is selected from the group consisting of



and



458. The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

459. The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage comprises a member selected from the group consisting of an olefinic bond at the alpha-position relative to the point of attachment to the nucleotide, a — CH_2NH— moiety, or both.

460. The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage comprises an allylamine group.

461. The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage comprises or includes an olefinic bond at the delta-position relative to the point of attachment to the nucleotide, or any of the moieties:

$$-CH{=}CH_2-NH-\ ,$$

$$-CH=CH-CH_2-NH-\ ,$$

$$-CH=CH-CH_2-O-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-NH-,$$

$$-S-,\ -\overset{\displaystyle O}{\overset{\|}{C}}-O,\text{ and } -O-\ .$$

462. The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage of Sig includes a glycosidic linkage moiety.

463. The oligo- or polydeoxyribonucleotide of claim 454, wherein said PM is a monophosphate, a diphosphate or a triphosphate and said Sig moiety is covalently attached to said PM through a phosphorus atom or phosphate oyxgen.

464. The oligo- or polydeoxyribonucleotide of claim 454, wherein Sig comprises a component selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme or an enzyme component, a hormone or a hormone component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten and an antibody or an antibody component, or a combination of any of the foregoing.

465. The oligo- or polydeoxyribonucleotide of claim 464, wherein said electron dense component comprises ferritin.

466. The oligo- or polydeoxyribonucleotide of claim 454, wherein Sig is complexed with a binding protein therefor, and said binding protein is conjugated to ferritin.

467. The oligo- or polydeoxyribonucleotide of claim 464, wherein said magnetic component comprises magnetic oxide.

468. The oligo- or polydeoxyribonucleotide of claim 467, wherein said magnetic oxide comprises ferric oxide.

469. The oligo- or polydeoxyribonucleotide of claim 464, wherein said enzyme or enzyme component is selected from the group consisting of alkaline phosphatase, acid phosphatase, β-galactosidase, ribonuclease, glucose oxidase and peroxidase.

470. The oligo- or polydeoxyribonucleotide of claim 464, wherein said metal-containing component is catalytic.

471. The oligo- or polydeoxyribonucleotide of claim 464, wherein said fluorescent component comprises a member selected from the group consisting of fluorescein, rhodamine and dansyl.

472. The oligo- or polydeoxyribonucleotide of claim 464, wherein Sig is selected from the group consisting of an antigen or hapten capable of complexing with an antibody or antibody component specific thereto, and an antibody or antibody component capable of complexing with an antigen or hapten.

473. The oligo- or polydeoxyribonucleotide of claim 454, wherein said oligo- or polydeoxyribonucleotide is terminally ligated or attached to a polypeptide.

474. A composition comprising the oligo- or polydeoxyribonucleotide of claim 454, a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed.

475. The composition of claim 474, wherein said polypeptide comprises polylysine.

476. The composition of claim 474, wherein said polypeptide is selected from the group consisting of avidin, streptavidin and anti-Sig immunoglobulin.

477. The composition of claim 474, wherein said Sig is a ligand and said polypeptide is an antibody thereto.

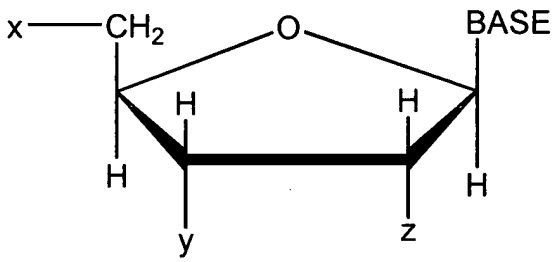
478. The oligo- or polydeoxyribonucleotide of claim 454, wherein said Sig moiety is attached to a terminal nucleotide in said oligo- or polydeoxyribonucleotide.

479. The oligo- or polydeoxyribonucleotide of claim 478, wherein the sugar moiety of said terminal nucleotide has a hydrogen atom at the 2' position thereof.

480. The oligo- or polydeoxyribonucleotide of claim 478, wherein the sugar moiety of said terminal nucleotide has hydrogen atoms at each of the 2' and 3' positions thereof.

481. The oligo- or polydeoxyribonucleotide of claim 454, comprising at least one ribonucleotide.

482. An oligo- or polydeoxyribonucleotide that is complementary to a nucleic acid of interest, or a portion thereof, comprising at least one modified nucleotide having the structural formula:



wherein BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, and wherein BASE is attached to the 1' position of the pentose ring from the N1 position when BASE is a pyrimidine or from the N9 position when BASE is a purine or a deazapurine;

wherein x is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate;

wherein y is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate;

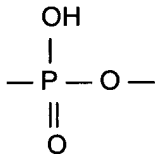
wherein z is H—; and

wherein Sig is covalently attached to x, y or z directly or through a chemical linkage, said Sig being a moiety capable of non-radioactive detection when so attached to x, y or z or when said oligo- or polydeoxyribonucleotide is hybridized to said complementary nucleic acid of interest, or portion thereof.

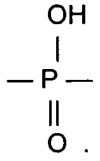
483. The oligo- or polydeoxyribonucleotide of claim 482, wherein said Sig is or renders the nucleotide or the oligo- or polydeoxyribonucleotide self-signaling or self-indicating or self-detecting.

484. The oligo- or polydeoxyribonucleotide of claim 482, wherein said Sig moiety comprises at least three carbon atoms.

485. The oligo- or polydeoxyribonucleotide of claim 482, wherein said covalent attachment is selected from the group consisting of



and

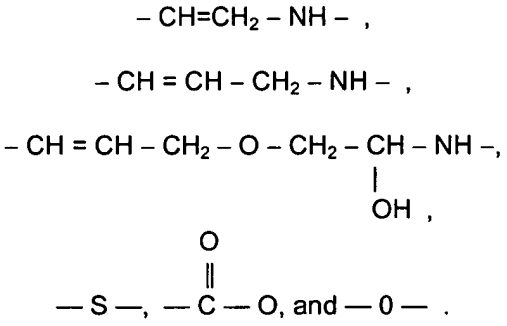


486. The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

487. The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage comprises a member selected from the group consisting of an olefinic bond at the alpha-position relative to the point of attachment to the nucleotide, a $-CH_2NH-$ moiety, or both.

488. The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage comprises an allylamine group.

489. The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage comprises or includes an olefinic bond at the delta-position relative to the point of attachment to x, y or z, or any of the moieties:



490. The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage of Sig includes a glycosidic linkage moiety.

491. The oligo- or polydeoxyribonucleotide of claim 482, wherein said x and y each comprise a member selected from the group consisting of a monophosphate, a diphosphate and a triphosphate and said Sig moiety is covalently attached to either or both of said x and y through a phosphorus atom or phosphate oyxgen.

492. The oligo- or polydeoxyribonucleotide of claim 482, wherein Sig comprises a component selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme or an enzyme component, a hormone or a hormone component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten and an antibody or an antibody component, or a combination of any of the foregoing.

493. The oligo- or polydeoxyribonucleotide of claim 492, wherein said electron dense component comprises ferritin.

494. The oligo- or polydeoxyribonucleotide of claim 482, wherein Sig is complexed with a binding protein therefor, and said binding protein is conjugated to ferritin.

495. The oligo- or polydeoxyribonucleotide of claim 492, wherein said magnetic component comprises magnetic oxide.

496. The oligo- or polydeoxyribonucleotide of claim 495, wherein said magnetic oxide comprises ferric oxide.

497. The oligo- or polydeoxyribonucleotide of claim 492, wherein said enzyme or enzyme component is selected from the group consisting of alkaline phosphatase, acid phosphatase, β-galactosidase, ribonuclease, glucose oxidase and peroxidase.

498. The oligo- or polydeoxyribonucleotide of claim 492, wherein said metal-containing component is catalytic.

499. The oligo- or polydeoxyribonucleotide of claim 492, wherein said fluorescent component comprises a member selected from the group consisting of fluorescein, rhodamine and dansyl.

500. The oligo- or polydeoxyribonucleotide of claim 492, wherein Sig is selected from the group consisting of an antigen or hapten capable of complexing with an antibody or antibody component specific thereto, and an antibody or antibody component capable of complexing with an antigen or hapten.

501. The oligo- or polydeoxyribonucleotide of claim 482, wherein said oligo- or polydeoxyribonucleotide is terminally ligated or attached to a polypeptide.

502. A composition comprising the oligo- or polydeoxyribonucleotide of claim 482, a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed.

503. The composition of claim 500, wherein said polypeptide comprises polylysine.

504. The composition of claim 502, wherein said polypeptide is selected from the group consisting of avidin, streptavidin and anti-Sig immunoglobulin.

505. The composition of claim 502, wherein said Sig is a ligand and said polypeptide is an antibody thereto.

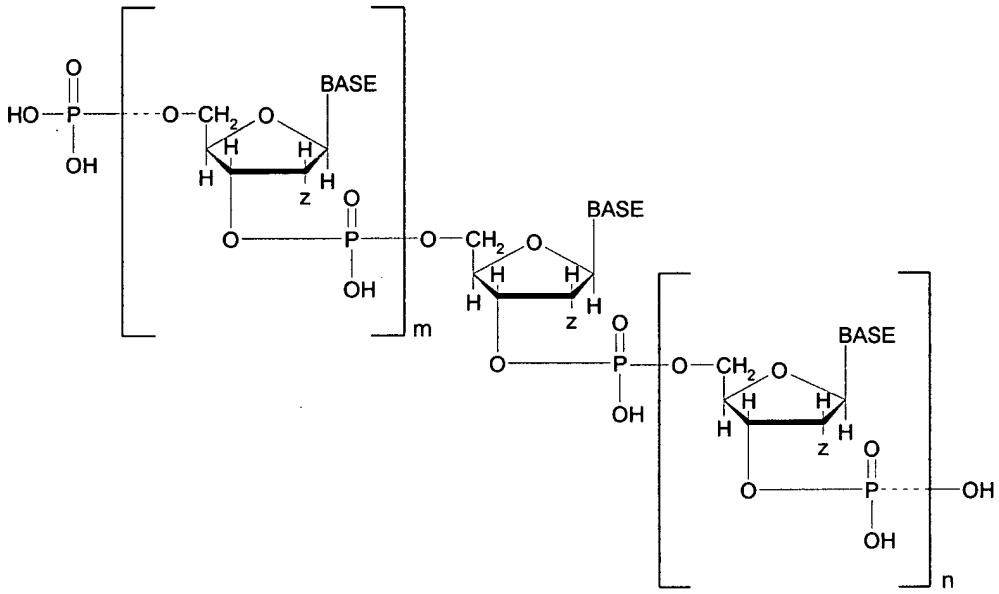
506. The oligo- or polydeoxyribonucleotide of claim 482, wherein said Sig moiety is attached to a terminal nucleotide in said oligo- or polydeoxyribonucleotide.

507. The oligo- or polydeoxyribonucleotide of claim 506, wherein z of said terminal nucleotide comprises a hydrogen atom at the 2' position thereof.

508. The oligo- or polydeoxyribonucleotide of claim 506, wherein both y and z of said terminal nucleotide comprise an oxygen atom at each of the 3' and 2' positions thereof, respectively.

509. The oligo- or polydeoxyribonucleotide of claim 482, comprising at least one ribonucleotide.

510. The oligo- or polydexoyribonucleotide of claim 482, having the structural formula:



wherein said Sig moiety is attached to at least one of the phosphate moieties in said structural formula.

511. An oligo- or polyribonucleotide comprising at least one ribonucleotide that is complementary to a nucleic acid of interest, or portion thereof, comprising at least one ribonucleotide having the formula

Sig – PM – SM – BASE

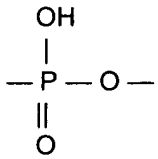
wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, said PM being attached to SM at a position of SM selected from the 2', 3' and 5' positions, or combinations thereof, said BASE being attached to SM, and Sig being covalently attached to PM directly or via a chemical linkage, said Sig being a moiety capable of non-radioactive detection when attached to PM, when said nucleotide is incorporated into said oligo- or polyribonucleotide, or when said oligo- or polyribonucleotide is hybridized to said

complementary nucleic acid, or portion thereof, provided that when Sig is attached through a chemical linkage to a terminal PM at the 3' position of a terminal ribonucleotide, said chemical linkage is not a cleaved 3' terminal ribonucleotide previously attached to said oligo- or polyribonucleotide.

512. The oligo- or polyribonucleotide of claim 511, wherein said Sig is or renders the nucleotide self-signaling or self-indicating or self-detecting.

513. The oligo- or polyribonucleotide of claim 511, wherein said Sig moiety comprises at least three carbon atoms.

514. The oligo- or polyribonucleotide of claim 511, wherein said covalent attachment is selected from the group consisting of



and

515. The oligo- or polyribonucleotide of claim 511, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

516. The oligo- or polyribonucleotide of claim 511, wherein said chemical linkage comprises a member selected from the group consisting of an olefinic bond at the alpha-position relative to the point of attachment to the nucleotide, a -CH_2NH- moiety, or both.

517. The oligo- or polyribonucleotide of claim 511, wherein said chemical linkage comprises an allylamine group.

518. The oligo- or polyribonucleotide of claim 511, wherein said chemical linkage comprises or includes an olefinic bond at the delta-position relative to the point of attachment to the nucleotide, or any of the moieties:

$$-CH{=}CH_2-NH-\ ,$$

$$-CH=CH-CH_2-NH-\ ,$$

$$-CH=CH-CH_2-O-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-NH-,$$

$$-S-,\quad -\overset{\displaystyle O}{\overset{\|}{C}}-O,\ \text{and}\ -O-\ .$$

519. The oligo- or polyribonucleotide of claim 511, wherein said chemical linkage of Sig includes a glycosidic linkage moiety.

520. The oligo- or polyribonucleotide of claim 511, wherein said PM is a monophosphate, a diphosphate or a triphosphate and said Sig moiety is covalently attached to said PM through a phosphorus atom or a phosphate oxygen.

521. The oligo- or polyribonucleotide of claim 511, wherein Sig comprises a component selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme or an enzyme component, a hormone or a hormone component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten and an antibody or an antibody component, or a combination of any of the foregoing.

522. The oligo- or polyribonucleotide of claim 521, wherein said electron dense component comprises ferritin.

523. The oligo- or polyribonucleotide of claim 511, wherein Sig is complexed with a binding protein therefor, and said binding protein is conjugated to ferritin.

524. The oligo- or polyribonucleotide of claim 521, wherein said magnetic component comprises a magnetic oxide.

525. The oligo- or polyribonucleotide of claim 524, wherein said magnetic oxide comprises ferric oxide.

526. The oligo- or polyribonucleotide of claim 521, wherein said enzyme or enzyme component is selected from the group consisting of alkaline phosphatase, acid phosphatase, β-galactosidase, ribonuclease, glucose oxidase and peroxidase.

527. The oligo- or polyribonucleotide of claim 521, wherein said metal-containing component is catalytic.

528. The oligo- or polyribonucleotide of claim 521, wherein said fluorescent component comprises a member selected from the group consisting of fluorescein, rhodamine and dansyl.

529. The oligo- or polyribonucleotide of claim 521, wherein Sig is selected from the group consisting of an antigen or hapten capable of complexing with an antibody or antibody component specific thereto, and an antibody or antibody component capable of complexing with an antigen or hapten.

530. The oligo- or polyribonucleotide of claim 511, wherein said oligo- or polyribonucleotide is terminally ligated or attached to a polypeptide.

531. A composition comprising the oligo- or polyribonucleotide of claim 511, a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed.

532. The composition of claim 531, wherein said polypeptide comprises polylysine.

533. The composition of claim 531, wherein said polypeptide is selected from the group consisting of avidin, streptavidin and anti-Sig immunoglobulin.

534. The composition of claim 531, wherein said Sig is a ligand and said polypeptide is an antibody thereto.

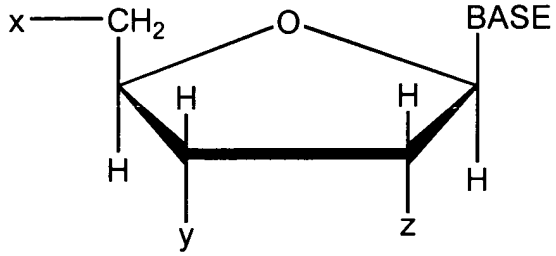
535. The oligo- or polyribonucleotide of claim 511, wherein said Sig moiety is attached to a terminal ribonucleotide in said oligo- or polyribonucleotide.

536. The oligo- or polyribonucleotide of claim 535, wherein the sugar moiety of said terminal ribonucleotide has a hydrogen atom at the 2' position thereof.

537. The oligo- or polyribonucleotide of claim 535, wherein the sugar moiety of said terminal nucleotide has a hydrogen atom at each of the 2' and 3' positions thereof.

538. The oligo- or polyribonucleotide of claim 511, comprising at least one deoxyribonucleotide.

539. An oligo- or polyribonucleotide that is complementary to a nucleic acid of interest, or portion thereof, comprising at least one nucleotide having the structural formula:



wherein BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, and wherein BASE is attached to the 1' position of the pentose ring from the N1 position when BASE is a pyrimidine or from the N9 position when BASE is a purine or a deazapurine;

wherein x is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate;

wherein y is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate;

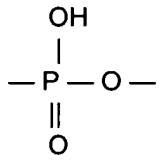
wherein z is HO— , and

wherein Sig is covalently attached to x, y or z directly or through a chemical linkage, said Sig being a moiety capable of non-radioactive detection when so attached to x, y or z, or when said oligo- or polyribonucleotide is hybridized to said nucleic acid of interest, or portion thereof, provided that when Sig is attached through a chemical linkage to y of a terminal ribonucleotide, said chemical linkage is a cleaved 3' terminal ribonucleotide previously attached to said oligo- or polyribonucleotide.

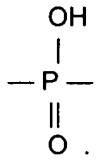
540. The oligo- or polyribonucleotide of claim 539, wherein said Sig is or renders the nucleotide or the oligo- or polynucleotide self-signaling or self-indicating or self-detecting.

541. The oligo- or polyribonucleotide of claim 539, wherein said Sig moiety comprises at least three carbon atoms.

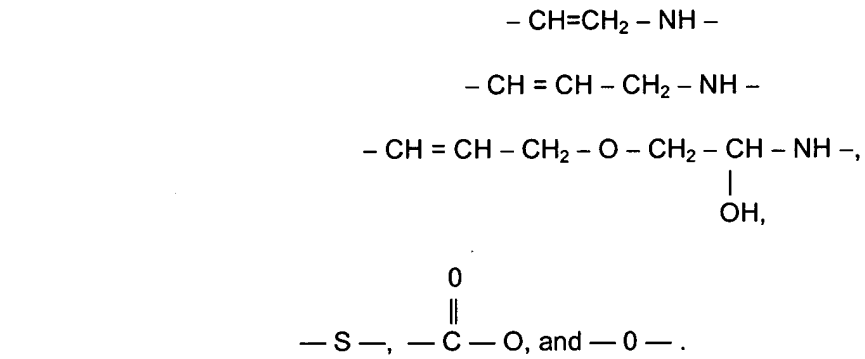
542. The oligo- or polyribonucleotide of claim 539, wherein said covalent attachment is selected from the group consisting of



and



546. The oligo- or polyribonucleotide of claim 539, wherein said chemical linkage comprises or includes an olefinic bond at the delta-position relative to x, y or z, or any of the moieties:



547. The oligo- or polyribonucleotide of claim 539, wherein said chemical linkage of Sig includes a glycosidic linkage moiety.

548. The oligo- or polyribonucleotide of claim 539, wherein said x and y each comprise a member selected from the group consisting of a monophosphate, a diphosphate and a triphosphate and Sig moiety is covalently attached to either or both of said x and y through a phosphorus atom or a phosphate oxygen.

549. The oligo- or polyribonucleotide of claim 539, wherein Sig comprises a component selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme or an enzyme component, a hormone or a hormone component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten and an antibody or an antibody component, or a combination of any of the foregoing.

550. The oligo- or polyribonucleotide of claim 549, wherein said electron dense component comprises ferritin.

551. The oligo- or polyribonucleotide of claim 539, wherein Sig is complexed with a binding protein therefor, and said binding protein is conjugated to ferritin.

552. The oligo- or polyribonucleotide of claim 549, wherein said magnetic component comprises magnetic oxide.

553. The oligo- or polyribonucleotide of claim 552, wherein said magnetic oxide comprises ferric oxide.

554. The oligo- or polyribonucleotide of claim 549, wherein said enzyme or enzyme component is selected from the group consisting of alkaline phosphatase, acid phosphatase, β-galactosidase, ribonuclease, glucose oxidase and peroxidase.

555. The oligo- or polyribonucleotide of claim 549, wherein said metal-containing component is catalytic.

556. The oligo- or polyribonucleotide of claim 549, wherein said fluorescent component comprises a member selected from the group consisting of fluorescein, rhodamine and dansyl.

557. The oligo- or polyribonucleotide of claim 549, wherein Sig is selected from the group consisting of an antigen or hapten capable of complexing with an antibody or antibody component specific thereto, and an antibody or antibody component capable of complexing with an antigen or hapten.

558. The oligo- or polyribonucleotide of claim 539, wherein said oligo- or polyribonucleotide is terminally ligated or attached to a polypeptide.

559. A composition comprising the oligo- or polyribonucleotide of claim 539, a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed.

560. The composition of claim 559, wherein said polypeptide comprises polylysine.

561. The composition of claim 559, wherein said polypeptide is selected from the group consisting of avidin, streptavidin and anti-Sig immunoglobulin.

562. The composition of claim 559, wherein said Sig is a ligand and said polypeptide is an antibody thereto.

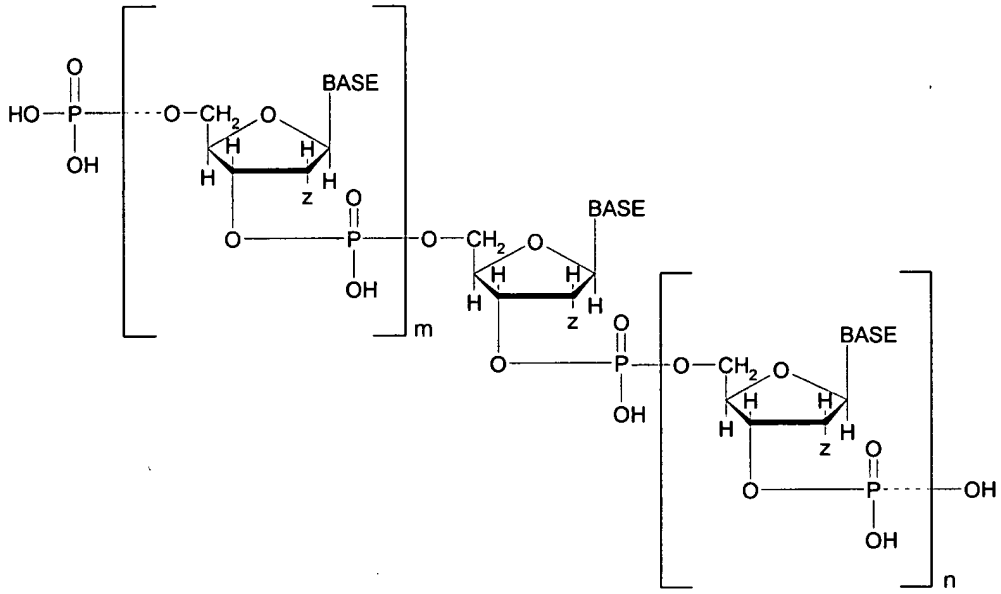
563. The oligo- or polyribonucleotide of claim 539, wherein said Sig moiety is attached to a terminal nucleotide in said oligo- or polyribonucleotide.

564. The oligo- or polyribonucleotide of claim 563, wherein z of said terminal nucleotide comprises a hydrogen atom at the 2' position thereof.

565. The oligo- or polyribonucleotide of claim 563, wherein both y and z of said terminal nucleotide comprise a hydrogen atom at each of the 3' and 2' positions thereof, respectively.

566. The oligo- or polyribonucleotide of claim 539, comprising at least one deoxyribonucleotide.

567. The oligo- or polyribonucleotide of claim 539, having the structural formula:



wherein said Sig moiety is attached to at least one of the phosphate moieties in said structural formula.

******

APPENDIX B
Unamended Claims in Appeal

454. An oligo- or polydeoxynucleotide comprising at least one modified nucleotide having the formula

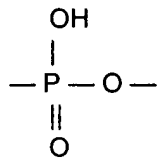
Sig—PM—SM—BASE

wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, said PM being attached to SM, said BASE being attached to SM, and Sig being covalently attached to PM directly or through a chemical linkage, said Sig being a moiety capable of non-radioactive detection when attached to PM or when said nucleotide is incorporated into said oligo- or polydeoxyribonucleotide.

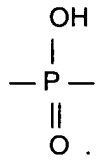
455. The oligo- or polydeoxyribonucleotide of claim 454, wherein said Sig is or renders the nucleotide self-signaling or self-indicating or self-detecting.

456. The oligo- or polydeoxyribonucleotide of claim 454, wherein said Sig moiety comprises at least three carbon atoms.

457. The oligo- or polydeoxyribonucleotide of claim 454, wherein said covalent attachment is selected from the group consisting of



and



458. The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

459. The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage comprises a member selected from the group consisting of an olefinic bond at the alpha-position relative to the point of attachment to the nucleotide, a — CH_2NH— moiety, or both.

460. The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage comprises an allylamine group.

461. The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage comprises or includes an olefinic bond at the delta-position relative to the point of attachment to the nucleotide, or any of the moieties:

$$-CH{=}CH_2-NH-\ ,$$

$$-CH=CH-CH_2-NH-\ ,$$

$$-CH=CH-CH_2-O-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-NH-,$$

$$-S-,\ -\overset{\displaystyle O}{\overset{\|}{C}}-O,\text{ and } -O-\ .$$

462. The oligo- or polydeoxyribonucleotide of claim 454, wherein said chemical linkage of Sig includes a glycosidic linkage moiety.

463. The oligo- or polydeoxyribonucleotide of claim 454, wherein said PM is a monophosphate, a diphosphate or a triphosphate and said Sig moiety is covalently attached to said PM through a phosphorus atom or phosphate oyxgen.

464. The oligo- or polydeoxyribonucleotide of claim 454, wherein Sig comprises a component selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme or an enzyme component, a hormone or a hormone component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten and an antibody or an antibody component, or a combination of any of the foregoing.

465. The oligo- or polydeoxyribonucleotide of claim 464, wherein said electron dense component comprises ferritin.

466. The oligo- or polydeoxyribonucleotide of claim 454, wherein Sig is complexed with a binding protein therefor, and said binding protein is conjugated to ferritin.

467. The oligo- or polydeoxyribonucleotide of claim 464, wherein said magnetic component comprises magnetic oxide.

468. The oligo- or polydeoxyribonucleotide of claim 467, wherein said magnetic oxide comprises ferric oxide.

469. The oligo- or polydeoxyribonucleotide of claim 464, wherein said enzyme or enzyme component is selected from the group consisting of alkaline phosphatase, acid phosphatase, β-galactosidase, ribonuclease, glucose oxidase and peroxidase.

470. The oligo- or polydeoxyribonucleotide of claim 464, wherein said metal-containing component is catalytic.

471. The oligo- or polydeoxyribonucleotide of claim 464, wherein said fluorescent component comprises a member selected from the group consisting of fluorescein, rhodamine and dansyl.

472. The oligo- or polydeoxyribonucleotide of claim 464, wherein Sig is selected from the group consisting of an antigen or hapten capable of complexing with an antibody or antibody component specific thereto, and an antibody or antibody component capable of complexing with an antigen or hapten.

473. The oligo- or polydeoxyribonucleotide of claim 454, wherein said oligo- or polydeoxyribonucleotide is terminally ligated or attached to a polypeptide.

474. A composition comprising the oligo- or polydeoxyribonucleotide of claim 454, a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed.

475. The composition of claim 474, wherein said polypeptide comprises polylysine.

476. The composition of claim 474, wherein said polypeptide is selected from the group consisting of avidin, streptavidin and anti-Sig immunoglobulin.

477. The composition of claim 474, wherein said Sig is a ligand and said polypeptide is an antibody thereto.

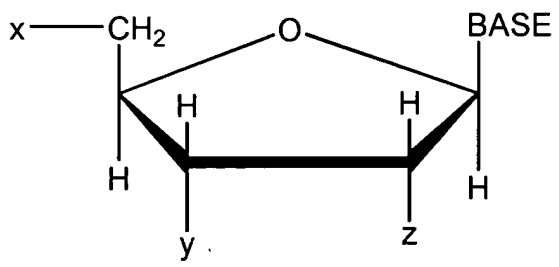
478. The oligo- or polydeoxyribonucleotide of claim 454, wherein said Sig moiety is attached to a terminal nucleotide in said oligo- or polydeoxyribonucleotide.

479. The oligo- or polydeoxyribonucleotide of claim 478, wherein the sugar moiety of said terminal nucleotide has a hydrogen atom at the 2' position thereof.

480. The oligo- or polydeoxyribonucleotide of claim 478, wherein the sugar moiety of said terminal nucleotide has hydrogen atoms at each of the 2' and 3' positions thereof.

481. The oligo- or polydeoxyribonucleotide of claim 454, comprising at least one ribonucleotide.

482. An oligo- or polydeoxyribonucleotide comprising at least one modified nucleotide having the structural formula:



wherein BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, and wherein BASE is attached to the 1' position of the pentose ring from the N1 position when BASE is a pyrimidine or from the N9 position when BASE is a purine or a deazapurine;

wherein x is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate;

wherein y is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate;

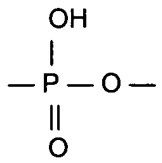
wherein z is H—; and

wherein Sig is covalently attached to x, y or z directly or through a chemical linkage, said Sig being a moiety capable of non-radioactive detection when so attached to x, y or z.

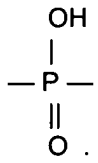
483. The oligo- or polydeoxyribonucleotide of claim 482, wherein said Sig is or renders the nucleotide or the oligo- or polydeoxyribonucleotide self-signaling or self-indicating or self-detecting.

484. The oligo- or polydeoxyribonucleotide of claim 482, wherein said Sig moiety comprises at least three carbon atoms.

485. The oligo- or polydeoxyribonucleotide of claim 482, wherein said covalent attachment is selected from the group consisting of



and



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486. The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

487. The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage comprises a member selected from the group consisting of an olefinic bond at the alpha-position relative to the point of attachment to the nucleotide, a $-CH_2NH-$ moiety, or both.

488. The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage comprises an allylamine group.

489. The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage comprises or includes an olefinic bond at the delta-position relative to the point of attachment to x, y or z, or any of the moieties:

$$-CH{=}CH_2-NH-\ ,$$

$$-CH=CH-CH_2-NH-\ ,$$

$$-CH=CH-CH_2-O-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-NH-,$$

$$-S-,\ -\overset{\displaystyle O}{\overset{\|}{C}}-O,\text{ and }-O-\ .$$

490. The oligo- or polydeoxyribonucleotide of claim 482, wherein said chemical linkage of Sig includes a glycosidic linkage moiety.

491. The oligo- or polydeoxyribonucleotide of claim 482, wherein said x and y each comprise a member selected from the group consisting of a monophosphate, a diphosphate and a triphosphate and said Sig moiety is covalently attached to either or both of said x and y through a phosphorus atom or phosphate oyxgen.

492. The oligo- or polydeoxyribonucleotide of claim 482, wherein Sig comprises a component selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme or an enzyme component, a hormone or a hormone component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten and an antibody or an antibody component, or a combination of any of the foregoing.

493. The oligo- or polydeoxyribonucleotide of claim 492, wherein said electron dense component comprises ferritin.

494. The oligo- or polydeoxyribonucleotide of claim 482, wherein Sig is complexed with a binding protein therefor, and said binding protein is conjugated to ferritin.

495. The oligo- or polydeoxyribonucleotide of claim 492, wherein said magnetic component comprises magnetic oxide.

496. The oligo- or polydeoxyribonucleotide of claim 495, wherein said magnetic oxide comprises ferric oxide.

497. The oligo- or polydeoxyribonucleotide of claim 492, wherein said enzyme or enzyme component is selected from the group consisting of alkaline phosphatase, acid phosphatase, β-galactosidase, ribonuclease, glucose oxidase and peroxidase.

498. The oligo- or polydeoxyribonucleotide of claim 492, wherein said metal-containing component is catalytic.

499. The oligo- or polydeoxyribonucleotide of claim 492, wherein said fluorescent component comprises a member selected from the group consisting of fluorescein, rhodamine and dansyl.

500. The oligo- or polydeoxyribonucleotide of claim 492, wherein Sig is selected from the group consisting of an antigen or hapten capable of complexing with an antibody or antibody component specific thereto, and an antibody or antibody component capable of complexing with an antigen or hapten.

501. The oligo- or polydeoxyribonucleotide of claim 482, wherein said oligo- or polydeoxyribonucleotide is terminally ligated or attached to a polypeptide.

502. A composition comprising the oligo- or polydeoxyribonucleotide of claim 482, a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed.

503. The composition of claim 500, wherein said polypeptide comprises polylysine.

504. The composition of claim 502, wherein said polypeptide is selected from the group consisting of avidin, streptavidin and anti-Sig immunoglobulin.

505. The composition of claim 502, wherein said Sig is a ligand and said polypeptide is an antibody thereto.

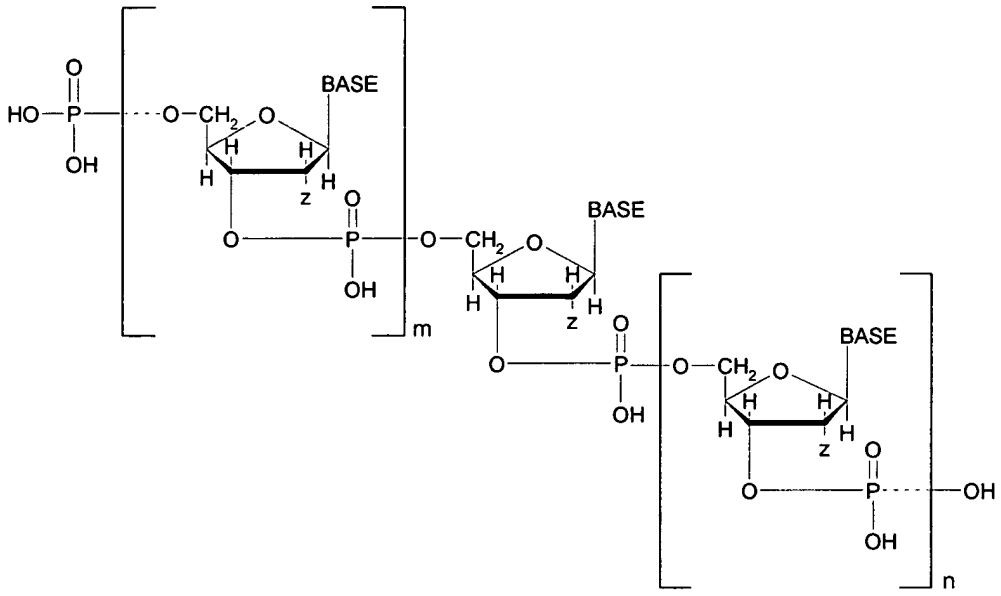
506. The oligo- or polydeoxyribonucleotide of claim 482, wherein said Sig moiety is attached to a terminal nucleotide in said oligo- or polydeoxyribonucleotide.

507. The oligo- or polydeoxyribonucleotide of claim 506, wherein z of said terminal nucleotide comprises a hydrogen atom at the 2' position thereof.

508. The oligo- or polydeoxyribonucleotide of claim 506, wherein both y and z of said terminal nucleotide comprise an oxygen atom at each of the 3' and 2' positions thereof, respectively.

509. The oligo- or polydeoxyribonucleotide of claim 482, comprising at least one ribonucleotide.

510. The oligo- or polydexoyribonucleotide of claim 482, having the structural formula:



wherein said Sig moiety is attached to at least one of the phosphate moieties in said structural formula.

511. An oligo- or polyribonucleotide comprising at least one ribonucleotide having the formula

Sig – PM – SM – BASE

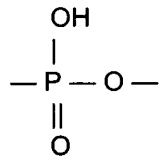
wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, said PM being attached to SM at a position of SM selected from the 2', 3' and 5' positions, or combinations thereof, said BASE being attached to SM, and Sig being covalently attached to PM directly or via a chemical linkage, said Sig being a moiety capable of non-radioactive detection when attached to PM or when said nucleotide is incorporated into said oligo- or polyribonucleotide, provided that when Sig is attached through a chemical linkage to

a terminal PM at the 3' position of a terminal ribonucleotide, said chemical linkage is not a cleaved 3' terminal ribonucleotide previously attached to said oligo- or polyribonucleotide.

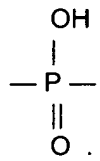
512. The oligo- or polyribonucleotide of claim 511, wherein said Sig is or renders the nucleotide self-signaling or self-indicating or self-detecting.

513. The oligo- or polyribonucleotide of claim 511, wherein said Sig moiety comprises at least three carbon atoms.

514. The oligo- or polyribonucleotide of claim 511, wherein said covalent attachment is selected from the group consisting of



and



515. The oligo- or polyribonucleotide of claim 511, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

516. The oligo- or polyribonucleotide of claim 511, wherein said chemical linkage comprises a member selected from the group consisting of an olefinic bond at the alpha-position relative to the point of attachment to the nucleotide, a $-CH_2NH-$ moiety, or both.

517. The oligo- or polyribonucleotide of claim 511, wherein said chemical linkage comprises an allylamine group.

518. The oligo- or polyribonucleotide of claim 511, wherein said chemical linkage comprises or includes an olefinic bond at the delta-position relative to the point of attachment to the nucleotide, or any of the moieties:

$$-CH{=}CH_2-NH-\ ,$$

$$-CH=CH-CH_2-NH-\ ,$$

$$-CH=CH-CH_2-O-CH_2-\underset{\displaystyle OH}{\underset{|}{CH}}-NH-,$$

$$-S-,\ -\overset{\displaystyle O}{\overset{\|}{C}}-O,\text{ and }-O-\ .$$

519. The oligo- or polyribonucleotide of claim 511, wherein said chemical linkage of Sig includes a glycosidic linkage moiety.

520. The oligo- or polyribonucleotide of claim 511, wherein said PM is a monophosphate, a diphosphate or a triphosphate and said Sig moiety is covalently attached to said PM through a phosphorus atom or a phosphate oxygen.

521. The oligo- or polyribonucleotide of claim 511, wherein Sig comprises a component selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme or an enzyme component, a hormone or a hormone component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten and an antibody or an antibody component, or a combination of any of the foregoing.

522. The oligo- or polyribonucleotide of claim 521, wherein said electron dense component comprises ferritin.

523. The oligo- or polyribonucleotide of claim 511, wherein Sig is complexed with a binding protein therefor, and said binding protein is conjugated to ferritin.

524. The oligo- or polyribonucleotide of claim 521, wherein said magnetic component comprises a magnetic oxide.

525. The oligo- or polyribonucleotide of claim 524, wherein said magnetic oxide comprises ferric oxide.

526. The oligo- or polyribonucleotide of claim 521, wherein said enzyme or enzyme component is selected from the group consisting of alkaline phosphatase, acid phosphatase, β-galactosidase, ribonuclease, glucose oxidase and peroxidase.

527. The oligo- or polyribonucleotide of claim 521, wherein said metal-containing component is catalytic.

528. The oligo- or polyribonucleotide of claim 521, wherein said fluorescent component comprises a member selected from the group consisting of fluorescein, rhodamine and dansyl.

529. The oligo- or polyribonucleotide of claim 521, wherein Sig is selected from the group consisting of an antigen or hapten capable of complexing with an antibody or antibody component specific thereto, and an antibody or antibody component capable of complexing with an antigen or hapten.

530. The oligo- or polyribonucleotide of claim 511, wherein said oligo- or polyribonucleotide is terminally ligated or attached to a polypeptide.

531. A composition comprising the oligo- or polyribonucleotide of claim 511, a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed.

532. The composition of claim 531, wherein said polypeptide comprises polylysine.

533. The composition of claim 531, wherein said polypeptide is selected from the group consisting of avidin, streptavidin and anti-Sig immunoglobulin.

534. The composition of claim 531, wherein said Sig is a ligand and said polypeptide is an antibody thereto.

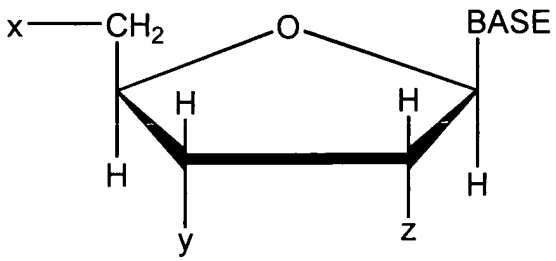
535. The oligo- or polyribonucleotide of claim 511, wherein said Sig moiety is attached to a terminal ribonucleotide in said oligo- or polyribonucleotide.

536. The oligo- or polyribonucleotide of claim 535, wherein the sugar moiety of said terminal ribonucleotide has a hydrogen atom at the 2' position thereof.

537. The oligo- or polyribonucleotide of claim 535, wherein the sugar moiety of said terminal nucleotide has a hydrogen atom at each of the 2' and 3' positions thereof.

538. The oligo- or polyribonucleotide of claim 511, comprising at least one deoxyribonucleotide.

539. An oligo- or polyribonucleotide comprising at least one nucleotide having the structural formula:



wherein BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, and wherein BASE is attached to the 1' position of the pentose ring from the N1 position when BASE is a pyrimidine or from the N9 position when BASE is a purine or a deazapurine;

wherein x is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate;

wherein y is selected from the group consisting of H— , HO— , a mono-phosphate, a di-phosphate and a tri-phosphate;

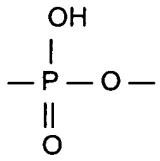
wherein z is HO— , and

wherein Sig is covalently attached to x, y or z directly or through a chemical linkage, said Sig being a moiety capable of non-radioactive detection when so attached to x, y or z, provided that when Sig is attached through a chemical linkage to y of a terminal ribonucleotide, said chemical linkage is a cleaved 3' terminal ribonucleotide previously attached to said oligo- or polyribonucleotide.

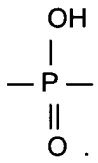
540. The oligo- or polyribonucleotide of claim 539, wherein said Sig is or renders the nucleotide or the oligo- or polynucleotide self-signaling or self-indicating or self-detecting.

541. The oligo- or polyribonucleotide of claim 539, wherein said Sig moiety comprises at least three carbon atoms.

542. The oligo- or polyribonucleotide of claim 539, wherein said covalent attachment is selected from the group consisting of



and

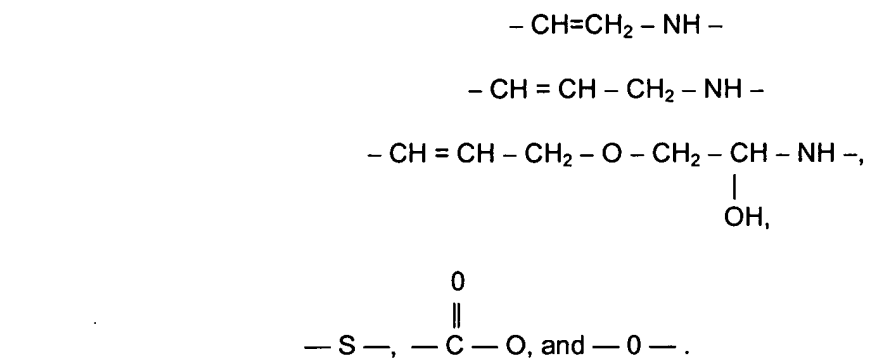


543. The oligo- or polyribonucleotide of claim 539, wherein said chemical linkage does not interfere substantially with the characteristic ability of Sig to form a detectable signal.

544. The oligo- or polyribonucleotide of claim 539, wherein said chemical linkage comprises a member selected from the group consisting of an olefinic bond at the alpha-position relative to the point of attachment to the nucleotide, a -CH_2NH- moiety, or both.

545. The oligo- or polyribonucleotide of claim 539, wherein said chemical linkage comprises an allylamine group.

546. The oligo- or polyribonucleotide of claim 539, wherein said chemical linkage comprises or includes an olefinic bond at the delta-position relative to x, y or z, or any of the moieties:



547. The oligo- or polyribonucleotide of claim 539, wherein said chemical linkage of Sig includes a glycosidic linkage moiety.

548. The oligo- or polyribonucleotide of claim 539, wherein said x and y each comprise a member selected from the group consisting of a monophosphate, a diphosphate and a triphosphate and Sig moiety is covalently attached to either or both of said x and y through a phosphorus atom or a phosphate oxygen.

549. The oligo- or polyribonucleotide of claim 539, wherein Sig comprises a component selected from the group consisting of biotin, iminobiotin, an electron dense component, a magnetic component, an enzyme or an enzyme component, a hormone or a hormone component, a metal-containing component, a fluorescent component, a chemiluminescent component, an antigen, a hapten and an antibody or an antibody component, or a combination of any of the foregoing.

550. The oligo- or polyribonucleotide of claim 549, wherein said electron dense component comprises ferritin.

551. The oligo- or polyribonucleotide of claim 539, wherein Sig is complexed with a binding protein therefor, and said binding protein is conjugated to ferritin.

552. The oligo- or polyribonucleotide of claim 549, wherein said magnetic component comprises magnetic oxide.

553. The oligo- or polyribonucleotide of claim 552, wherein said magnetic oxide comprises ferric oxide.

554. The oligo- or polyribonucleotide of claim 549, wherein said enzyme or enzyme component is selected from the group consisting of alkaline phosphatase, acid phosphatase, β-galactosidase, ribonuclease, glucose oxidase and peroxidase.

555. The oligo- or polyribonucleotide of claim 549, wherein said metal-containing component is catalytic.

556. The oligo- or polyribonucleotide of claim 549, wherein said fluorescent component comprises a member selected from the group consisting of fluorescein, rhodamine and dansyl.

557. The oligo- or polyribonucleotide of claim 549, wherein Sig is selected from the group consisting of an antigen or hapten capable of complexing with an antibody or antibody component specific thereto, and an antibody or antibody component capable of complexing with an antigen or hapten.

558. The oligo- or polyribonucleotide of claim 539, wherein said oligo- or polyribonucleotide is terminally ligated or attached to a polypeptide.

559. A composition comprising the oligo- or polyribonucleotide of claim 539, a polypeptide capable of forming a complex with Sig and a moiety which can be detected when such complex is formed.

560. The composition of claim 559, wherein said polypeptide comprises polylysine.

561. The composition of claim 559, wherein said polypeptide is selected from the group consisting of avidin, streptavidin and anti-Sig immunoglobulin.

562. The composition of claim 559, wherein said Sig is a ligand and said polypeptide is an antibody thereto.

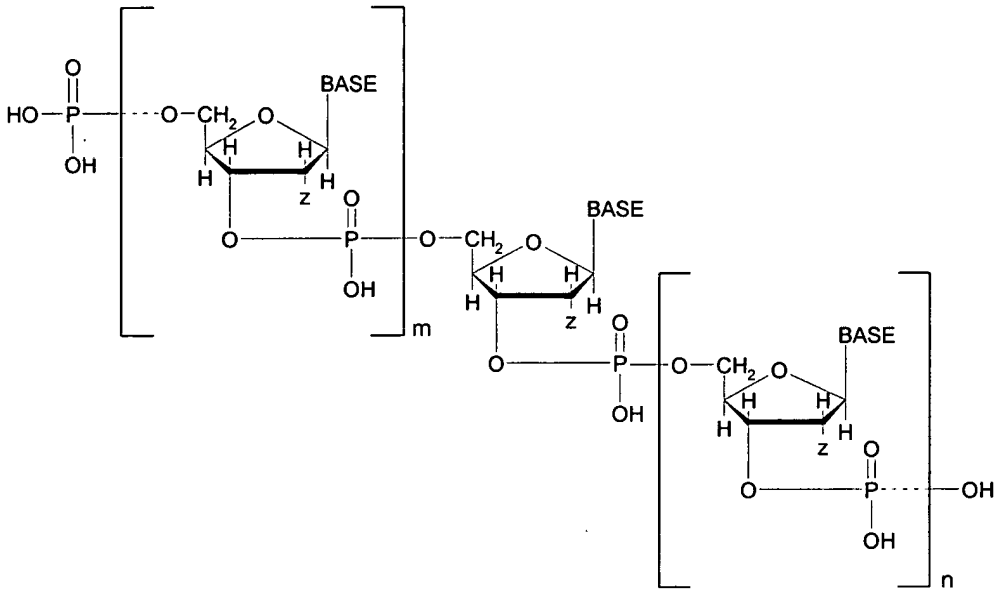
563. The oligo- or polyribonucleotide of claim 539, wherein said Sig moiety is attached to a terminal nucleotide in said oligo- or polyribonucleotide.

564. The oligo- or polyribonucleotide of claim 563, wherein z of said terminal nucleotide comprises a hydrogen atom at the 2' position thereof.

565. The oligo- or polyribonucleotide of claim 563, wherein both y and z of said terminal nucleotide comprise a hydrogen atom at each of the 3' and 2' positions thereof, respectively.

566. The oligo- or polyribonucleotide of claim 539, comprising at least one deoxyribonucleotide.

567. The oligo- or polyribonucleotide of claim 539, having the structural formula:



wherein said Sig moiety is attached to at least one of the phosphate moieties in said structural formula.

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